When citing an abstract from the 2015 annual meeting please use the format below.

[Authors]. [Abstract Title]. Program No. XXX.XX. 2015 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2015. Online.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.01/A1

Topic: A.02. Postnatal Neurogenesis

Support: NSFC 31371101

NSFC 31070955

NSFC 81171152

Title: Plastic changes of the early spinal cord in mice with the genetic absence of corticospinal tract

Authors: ***J. DUAN**, L. HUANG, Y. QU, L. ZHOU;
Guangdong-Hongkong-Macau Inst. of CNS Regeneration, Jinan Univ., Jinan Univ., Guangdong, China

Abstract: Spinal cord maturation is followed with the corticospinal inputs. In order to understand how much the early events in the spinal cord are influenced by the arrival of corticopsinal axons, we studied the monosynaptic elimination, the expression of activity-related genes, neurotrophins and their receptors from postnatal day (P) 0 to 21, using Celsr3|Emx1 mice, in which corticospinal axons never reach the spinal cord. The corticospinal tract was specifically labeled by anti-PKCγ antibody in the dorsal funiculus, showing gradually increased from P0 to P21 in the control, but never visible in the mutant. Using anti-parvalbumin and ChAT double immunostaining, close contacts of proprioceptive fibers and spinal motoneurons appeared at P0 and were gradually eliminated thereafter, showing no difference between control and mutant mice. The number of parvalbumin-positive interneurons behaved an increased trend from P7 to P21 and an elevation of c-Jun protein was seen at P7, but there was no significant difference at each time-point between two groups. In control samples, CNTF protein was increased at P7 and gradually decreased after P14, which fluctuation from P0 to P14 was not found in mutant samples, and reached comparable levels at P14 and P21 between two groups. The expression patterns of NT3, truncated and full-length TrkC were similar in the control: increasing at P7 and decreasing after P14. In contrast, a significant increase of these neurotrophins was not found at P7 in the mutant. The similar fluctuation of BDNF, TrkB , GDNF and p75NTR proteins was present in two genotypes, such as BDNF increased at P7 and decreased at P14, the ratio of truncated to full-length TrkB increased after P14, GDNF increased at P14, and p75NTR gradually decreased after P7. In conclusion, the elimination of monosynaptic contacts and the changes of the expression of activity-related genes, neurotrophins and their receptors happen during the critical period, but these events are not highly dependent on the corticospinal inputs, except for the expression of CNTF, NT3 and TrkC.

Disclosures: **J. Duan:** None. **L. Huang:** None. **Y. Qu:** None. **L. Zhou:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.02/A2

Topic: A.02. Postnatal Neurogenesis

Support: Wesleyan University

Title: Incorporation of parvalbumin expressing neurons in the caudomedial nidopallium (NCM) of juvenile zebra finches is affected by song tutor availability

Authors: ***K. ASIK**, J. R. KIRN;
Biol. Dept., Wesleyan Univ., Middletown, CT

Abstract: The songbird telencephalon continually receives new neurons throughout life. Peak levels of new neuron incorporation in HVC (proper name) have been associated with a critical period for vocal learning (Nordeen & Nordeen, 1988.). A subsequent, age-related drop in the rate of new neuron incorporation is accompanied by song crystallization and the end of the critical period for song learning. The caudomedial nidopallium (NCM), analogous to mammalian auditory association cortex, is a crucial brain region for acquiring tutor song memory (Bolhuis & Gahr, 2006). Additionally, NCM contains a high number of inhibitory interneurons, including parvalbumin-expressing (PV) interneurons, which are known to be important for proper development of many critical period processes across several organisms (Pinaud et al., 2004, Phan et al., 2006, Hensch, 2005). The extents to which levels of NCM neuron addition relate to learning have not been explored. We injected male zebra finches with the cell birth marker Bromodeoxyuridine (BrdU) during song learning to quantify new NCM neurons including those expressing parvalbumin. We found a peak rate of incorporation of both new PV and new non-PV neurons around 40 days-post-hatch (dph) in normally raised males, which is consistent with the timeline of tutor song memory acquisition. For both types of new neurons, this peak was followed by a decline at around 60 dph and a further decline in adulthood. Another group of zebra finches was raised without tutors, a procedure that delays the normal end of the critical period (Eales, 1985). We found elevated levels of both PV and non-PV new neurons at around 60 dph, compared to normally raised birds. These findings suggest a relationship between levels of new neuron incorporation and the timing of song learning, a crucial process for mating success in male zebra finches.

Disclosures: **K. Asik:** None. **J.R. Kirn:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.03/A3

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant DC012567

Title: Odorant receptor expression is perturbed in mice following recovery from genetically-mediated lesion

Authors: *J. H. BRANN[1], X. ZHANG[2], E. F. SPINAZZI[3], C. FRANKLIN[1], P. LIS[1], N. KHARAS[3], C. ALTOMARE[3], S. J. FIRESTEIN[3];
[1]Dept. Biol., Loyola Univ. Chicago, Chicago, IL; [2]BioInfoRx, Madison, WI; [3]Dept. Biol. Sci., Columbia Univ., New York, NY

Abstract: Several repositories of neural stem cells are resident in the nervous system. One population, residing in the olfactory epithelium (OE) lining the nasal cavity, generates excitatory projection neurons that extend a long axon from the neuroepithelium to the olfactory bulb. Each sensory neuron expresses a single odorant receptor (OR), a single gene choice out of ~1200 OR genes in mice, conferring an identity required for odorant detection and appropriate targeting of its axon to the olfactory bulb. Basal stem cells have been known for more than 30 years to generate sensory neurons in the young adult OE, but the ability of their counterparts in aged tissue to recapitulate the OE is relatively unexplored. In particular, aging may alter OR expression, implying receptor gene choice in newborn neurons may become restricted. Here we probe the ability of the stem cell to generate a diverse array of sensory neurons expressing the appropriate repertoire of odorant receptors in aged animals. To this end we generated a line of mice, iDTR+OMPCre/+, whereby a Cre-mediated excision of a STOP cassette renders mature neurons sensitive to diphtheria toxin (DT) via activation of the DT receptor. This method permits a specific and reversible ablation of mature (OMP-expressing) neurons upon DT administration but without damage to potential synaptic targets in the OB or to other cell types found in the OE. We administered either DT or saline to male mice of several age groups (2-18 months) for six days. RNAs were harvested 30 days following ablation, to allow for complete degeneration and subsequent recovery of the OE. Results reveal that age does not affect the cohort of OR genes expressed following recovery from lesion in DT injected mice. In addition we observe that age does not affect OR expression in saline injected mice. However, the OR repertoire does significantly change following ablation and these effects are observed at all ages tested. These results provide evidence that the regenerative potential of the neuronal stem cell is not altered by age per se, as a wide array of sensory neurons are generated. However, lesion induced ablation of a large number of sensory neurons disrupts the typical OR expression patterns observed in intact mice.

Disclosures: **J.H. Brann:** None. **X. Zhang:** None. **E.F. Spinazzi:** None. **C. Franklin:** None. **P. Lis:** None. **N. Kharas:** None. **C. Altomare:** None. **S.J. Firestein:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.04/A4

Topic: A.02. Postnatal Neurogenesis

Support: ANR France

FRM France

Jerome Lejeune France

LABEX SIGNALIFE France

Title: A novel postnatal population of cortical projection neurons characterized by the co-expression of Ctip2 and Satb2 in the mouse neocortex

Authors: ***M. C. STUDER**[1], K. HARB[1], E. MAGRINELLI[1], C. NICOLAS[1], N. LUKIANETS[1], G. SANDOZ[1], F. GRAMMONT[2], C. ALFANO[1];
[1]Inst. of Biology, Ibv (UMR INSERM1091/CNRS7277/UNS), Nice, France; [2]Lab. J.A. Dieudonné (UMR CNRS 7351), Univ. of Nice Sophia Antipolis, Nice, France

Abstract: The mammalian neocortex is tangentially subdivided into several functional areas and radially organized into six neuronal layers showing prominent diversities in features and functions despite similarities in their laminar organization. Differences in the molecular identity, morphology and long-range connectivity of residing neurons allow neuronal diversity of the mature cortex. The specification and differentiation of long-distance neocortical projection neurons are first controlled by the expression of transcription factors, which become gradually restricted and mutually exclusive during development. However, little is known on the mechanisms by which area-specific projection neurons acquire their subtype identity and final connectivity after birth. In this study, we show that neurons co-expressing Ctip2 and Satb2, respectively involved in the early specification of subcerebral (SCPN) and callosal projection neurons (CPN), progressively increase in numbers between P0 and P21 in the mouse somatosensory cortex. This neuron population displays unique connectivity and morphology features, distinct molecular codes and electrophysiological properties. Since it is known that Satb2 inhibits the expression of Ctip2 during development, we also investigated the molecular mechanisms allowing their co-expression at postnatal stages and in different neocortical areas. Our study thus demonstrates that co-expression of mutually exclusive CPN- and SCPN-specific

determinants enhances heterogeneity of distinct neuronal subpopulations and thereby subsequent complexity of the postnatal mammalian neocortex.

Disclosures: **M.C. Studer:** None. **K. Harb:** None. **E. Magrinelli:** None. **C. Nicolas:** None. **N. Lukianets:** None. **G. Sandoz:** None. **F. Grammont:** None. **C. Alfano:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.05/A5

Topic: A.02. Postnatal Neurogenesis

Support: NIH-NIDCD 011137

Title: Differential effects on ventricular zone cell genesis following deafferentation or direct injury to the olfactory bulb in adult zebrafish

Authors: *D. M. TRIMPE, C. A. BYRD-JACOBS;
Western Michigan Univ., Kalamazoo, MI

Abstract: Stem cells in the telencephalic ventricular zone generate neural precursor cells for the olfactory bulb that are involved in maintenance and repair following injury. We have previously shown that the adult zebrafish olfactory bulb is able to regenerate following deafferentation and direct injury. Here, we examine the potential differential effects of injury type on cell genesis in the ventricular zone. The hypothesis of this study was that the ventricular zone will exhibit a similar injury response to various forms of damage in which cell genesis will increase in the ventricular zone ipsilateral to the injury site, with an incremental return of cell genesis to control levels following recovery. Temporary, partial deafferentation of the olfactory bulb was achieved by single or repeated intranasal irrigation with Triton X-100; complete, permanent deafferentation was accomplished with cautery ablation of the olfactory organ; and direct injury involved a stab wound to the bulb. The potential effects on cell proliferation were examined using intraperitoneal injection of bromodeoxyuridine (BrdU). A single application of Triton X-100 caused a significant bilateral increase in proliferating cells in deafferented fish. Three weeks of deafferentation with Triton X-100 significantly increased the number of anti-BrdU labeled profiles in the treated side, whereas the untreated side had returned to control levels. With 4 weeks of Triton X-100 deafferentation, both sides appeared to exhibit reduced cell genesis but were not significantly different from control levels. Similarly, ventricular zone proliferation following complete, permanent deafferentation exhibited a bilateral increase anti-BrdU staining intensity suggesting enhanced proliferation. Fish examined 4h following direct injury showed a significant increase in anti-BrdU labeled profiles on the treated side only, while at 24h post-lesion, anti-BrdU labeled profiles remained significantly increased on the treated side but were

significantly reduced on both sides compared to control fish. Ventricular zone response during recovery from temporary deafferentation was then examined at 1 week and 3 weeks following 3 weeks of Triton X-100 deafferentation. One week of recovery resulted in a significant bilateral decrease in anti-BrdU labeled profiles, while 3 weeks of recovery allowed both sides to return to control levels. Thus, contrary to our hypothesis, these findings suggest that cell genesis in the ventricular zone exhibits an injury-specific response where deafferentation results in bilateral changes and direct injury causes primarily ipsilateral effects in cell proliferation.

Disclosures: D.M. Trimpe: None. **C.A. Byrd-Jacobs:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.06/A6

Topic: A.02. Postnatal Neurogenesis

Title: Spatiotemporal dynamics of adult-born granule cell mossy fiber terminal development in aged hippocampus

Authors: K. D. MURRAY[1], X.-B. LIU[3], L. P. CAMERON[1], M. YOSHIHARA[1], *H.-J. CHENG[2];
[1]Ctr. for Neurosci., [2]UC Davis, Davis, CA; [3]Cell and Human Anat., Univ. of California, Davis, Davis, CA

Abstract: The constitutive generation of new neurons is now a well-established feature of the adult mammalian brain. A central question regarding adult neurogenesis is how new neurons integrate into existing circuitry particularly in aged brains where neurogenesis has been reported to significantly decline. To address this we combined a cellular morphometric analyses with serial immune-EM ultra-structural analysis in a conditional transgenic mouse line to measure the spatiotemporal dynamics of adult-born hippocampal mossy fiber (MF) synaptic development in young adult (3 months, 3M) and aged (18 month, 18M) brains. We used the Gli1CreER tamoxifen (TM) inducible reporter line to fate map newborn granule cells and their processes in young as well as aged brains. We found that while proliferation and survival of newborn neurons decreases with age in the short term (4 weeks (W) post TM), in the long term (16W post TM) no difference was observed between young adult and aged brains. This suggests robust long term integration in newborn neurons in aged brain. However, differences in spatiotemporal dynamics of newborn MF development were observed. In 3M hippocampus between 4W and 16W post TM the density of newly formed MF terminals decreased while the average size of their boutons increased. In contrast, in the 18M hippocampus MF bouton density steadily increased during development, while average bouton size decreased. EM analysis confirmed these developmental trajectories and revealed differences in the pattern of structural maturation in young adult and

aged brains. In particular, in young adult hippocampus newborn MF boutons initially form nascent synaptic contacts that mature from 4W to 16W post TM while in aged hippocampus newborn MF boutons were fully mature from the first times examined (4W post TM). We also observed a significant amount of terminal "sharing" between newborn MF terminals and pre-existing terminals in young adult brain during the entire developmental period examined (4-16W post TM), but could only observed similar sharing at young developmental times (4W post TM) in aged hippocampus. Together these data suggest that the mechanism by which newborn hippocampal GCs integrate their axons into mature circuits is fundamentally different in young adult brains and aged hippocampi. In young adult brains new MF terminals are added which compete with pre-existing terminals for target space in mature circuits while in aged brain they replace existing circuits. Therefore the cognitive impact of adult neurogenesis could be profoundly different in young adult versus aged brain.

Disclosures: K.D. Murray: None. **X. Liu:** None. **L.P. Cameron:** None. **M. Yoshihara:** None. **H. Cheng:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.07/A7

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01 NS080979

NIH Grant P30 NS061800

Ellison Medical Foundation

Title: Preferential targeting of lateral perforant path inputs to newly-generated dentate granule cells

Authors: N. I. WOODS, C. CHATZI, J. PEREDERIY, K. TOVAR, *G. L. WESTBROOK; Vollum Inst., Portland, OR

Abstract: Primary and associative sensory information converge in the entorhinal cortex before entering the hippocampus via the perforant path. Lateral and medial entorhinal neurons project axons to the dentate gyrus as discrete laminae, providing segregated functional and anatomic innervation of granule cell dendrites in the molecular layer. To examine the distribution of perforant path inputs onto adult-generated newborn granule cells, we selectively activated or silenced inputs from the entorhinal cortex. Following labeling of lateral or entorhinal cortex in young adult mice with AAV9-CAG- channelrhodopsin2-GFP, we optogenetically stimulated either lateral or medial entorhinal axons in brain slices, and verified laminar-specific activation

of mature dentate granule cells. We then labeled newly-generated granule cells with a retrovirus to determine the distribution of perforant path inputs onto newborn neurons. At 21 days post-injection, optogenetic stimulation revealed that excitatory inputs onto newborn neurons were almost exclusively associated with lateral perforant path axons that target dendrites in the outer molecular layer. Although the medial perforant path contributed only a minor component to the excitatory input to newborn neurons, chronic silencing of the medial perforant path with AAV9-expressing tetanus toxin, disrupted the development of excitatory inputs onto newborn neurons (but not mature dentate granule cells) as measured by EPSC amplitude and dendritic spine number. Our results indicate that newborn neurons are preferentially targeted by perforant path axons from the lateral entorhinal cortex, which carry associative (contextual) information - a distribution pattern consistent with a role for newborn neurons in pattern separation during memory formation.

Disclosures: N.I. Woods: None. **C. Chatzi:** None. **J. Perederiy:** None. **K. Tovar:** None. **G.L. Westbrook:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.08/A8

Topic: A.02. Postnatal Neurogenesis

Support: Program Revenue

Title: Combining Ki-67 and the thymidine analogue 5-chloro-2-deoxyuridine (CldU) to monitor cell proliferation in the dentate gyrus of the adult rat

Authors: *R. E. KALIL, M. L. HENDRICKSON, L. R. STIPPICH;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: The protein Ki-67 is widely used as a marker of cell proliferation. Despite its frequent use, the *in-vivo* half-life of Ki-67 is still unknown, although most agree that it is probably brief. If the half-life of Ki-67 were known, Ki-67 could be used accurately as a marker for cell proliferation in studies of neurogenesis in the adult mammalian hippocampus to determine whether individual dividing cells continue to proliferate. To investigate the half-life of Ki-67 we analyzed cell proliferation in the subgranular zone (SGZ) of the dentate gyrus in adult male Sprague Dawley rats. In addition, in order to monitor the temporal distribution of proliferating cells, one injection of CldU (42.5 mg/kg) was administered and the animals then were permitted to survive for 30 minutes, 4 hours, 8 hours, 24 hours, 48 hours, 1 week, and 3 weeks before being deeply anesthetized and perfused with 4% paraformaldehyde in phosphate buffer. Following perfusion, brains were sectioned through the dentate gyrus at 40 μm and stained with

antibodies for Ki-67 and CldU. A rabbit monoclonal anti-Ki-67 primary antibody and a donkey anti-rabbit secondary antibody conjugated to the fluorophore Cy-2 were used to resolve cells expressing Ki-67. Similarly, a sheep polyclonal anti-BrdU primary antibody and a donkey anti-sheep secondary antibody conjugated to the fluorophore Cy-3 were used to resolve cells positive for CldU. DAPI was used to stain all cell nuclei in order to clearly delineate cells in the SGZ stained for Ki-67 and CldU. Cells expressing Ki-67 were regularly seen in clusters of 2 to 5 cells. Cells that had incorporated CldU were typically observed in groups of 2 to 3 cells at survival periods that ranged from 30 minutes to 48 hours and as isolated cells at 1 and 3 week intervals. At survival periods of 30 minutes, 4 hours, and 8 hours we observed a near-complete overlap of staining for CldU and Ki-67 in SGZ cells. At survival periods greater than 8 hours, the overlap in staining is still observed, albeit decreased, suggesting that the half-life of Ki-67 may be longer than results from *in vitro* experiments indicate.

Disclosures: **R.E. Kalil:** None. **M.L. Hendrickson:** None. **L.R. Stippich:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.09/A9

Topic: A.02. Postnatal Neurogenesis

Support: ARSEP

ELA

Title: Adult peripheral nervous system stem cells: from their identification towards their role and fate in pathological conditions

Authors: *M. MANIGLIER[1], M. VIDAL[2], C. BACHELIN[2], C. DEBOUX[2], A. BARON VAN EVERCOOREN[2];
[1]ICM (institut Du Cerveau Et De La Moelle Epiniere, Paris, France; [2]ICM, INSERM U1127, UPMC Univ. Paris 06 UM 75, CNRS UMR 7225, Paris, France

Abstract: Neuropathic pain (NP) is a major public health problem caused by a primary lesion or dysfunction in the nervous system, with no effective therapy available. Since dorsal root ganglion (DRG) is considered as an active player in the development and maintenance of NP, elucidate DRG cellular pain partners is crucial to find new molecular targets. Prior to the conception of these new strategies, it is necessary to identify and characterize the different DRG cells to understand their involvement in pain. Recent publications suggested the presence of stem cells in the adult DRG. So far, these cells were not examined in NP studies due to the lack of tools to correctly identify and track them *in situ*. Using newly developed powerful transgenic mice combined with 5-ethynyl-2'-deoxyuridine incorporation assay, immunohistochemistry or

electron microscopy, we were able to identify different subset of cells with fast proliferating rate and one type of slowly proliferating stem cells. These adult DRG stem cells were purified to study specifically their properties *in vitro*. FACS sorted positive stem cells had a slower expansion rate, generated spheres over time with neurogenic and gliogenic potentials. Finally, using lineage tracing with an inducible mouse line to activate YFP expression in adult DRG stem cells and their progeny, we are able to investigate their fate and role in a DRG neurotoxic model. By combining multiple approaches, we succeeded to identify DRG stem cells, highlight their specific properties by comparing them to adult central nervous system stem cells and test their participation to DRG neurogenesis in pathophysiological conditions. This fundamental knowledge on adult PNS stem cells will open new avenues to therapeutic strategies in NP. Support by ARSEP and ELA.

Disclosures: **M. Maniglier:** None. **M. Vidal:** None. **C. Bachelin:** None. **C. Deboux:** None. **A. Baron Van Evercooren:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.10/A10

Topic: A.02. Postnatal Neurogenesis

Support: NIH Intramural Research Program

Title: Effects of cholinergic manipulations on adult neurogenesis

Authors: ***S. L. OTTO**, J. L. YAKEL;
Neurobio. Lab. - Ion Channel Physiol. Group, NIEHS, Research Triangle Park, NC

Abstract: Adult neurogenesis is a life-long process whereby neural stem cells located in the subgranular zone of the dentate gyrus in both humans and rodents, as well as in the subventricular zone in rodents, divide, differentiate, mature and integrate in the local circuitry. While the signaling important in each of these stages is still poorly understood, it is known that local cholinergic signaling plays a role in the subventricular zone. The dentate gyrus receives cholinergic input from the basal forebrain, a potential source of signaling that may impact adult neurogenesis. Both metabotropic and ionotropic cholinergic receptors could contribute a counterbalanced set of signals to modulate neurogenesis. For example, α7 nicotinic acetylcholine receptors (nAChRs) are considered important in maturation of granular cells in the dentate gyrus and have been shown to be expressed on cells in the subgranular zone. α7 nAChRs are highly expressed in neonates and then see declining levels of expression until reaching adult levels at 3 weeks in mice. Though environmental factors as diverse as exercise and solitude have been shown to have an effect on adult neurogenesis, extant literature conflicts as to possible

cholinergic effects. Little is known of the possible mechanism(s) through which cholinergic signaling may act. To better understand adult neurogenesis in the dentate gyrus, we use a NestinCrERT2 mouse that permits the labeling of Nestin+ neural stem cells. Using this model we show that expression of Nestin+ neural stem cells in juvenile mice (p21-23) is unaffected by exposure to nicotine during gestation and nursing, however is significantly decreased by exposure to high levels of choline, which is known to bind to α7 nAChRs. On the other hand intermediate precursor cells are unaffected. By contrast in adults (8-10 weeks), exposure to the choline diet during gestation and nursing had no lasting effect, but chronic nicotine exposure significantly reduced levels of Nestin+ neural stem cells. In addition, a subset (approximately 50%) of all animals exposed to nicotine at any age showed an increased level of granular cells that were Nestin+, possibly indicating that nicotine exposure is increasing the rate at which stem cells mature. While preliminary, these studies show that the effects on neurogenesis of cholinergic signaling may vary with the age of the animal. Ongoing research is attempting to envision cholinergic signaling in organotypic culture, correlate changes in numbers of Nestin+ cells with neurogenesis, quantitate the effect of α7 nAChR knockout on adult neurogenesis, and study the Nestin+ granular cells seen after exposure to nicotine.

Disclosures: S.L. Otto: None. **J.L. Yakel:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.11/A11

Topic: A.02. Postnatal Neurogenesis

Support: NSC 101-2314-B-004-001

Title: Survival of adult born hippocampal neurons

Authors: P.-J. WU[1], H.-H. LIN[1], *G.-J. LAI[1,2];
[1]Natl. Chengchi Univ., Taipei City, Taiwan; [2]Res. center for mind,brain & learning, Natl. Chengchi Univ., Taipei, Taiwan

Abstract: The hippocampus is a brain region critical to learning and memory and is a frequent target of many neurological diseases such as Alzheimer's, other forms of dementia, and chronic stress that have dramatic cognitive consequences. The Subgranular zone (SGZ) of the hippocampus is one of the mammalian brain regions where new neurons are generated continuously throughout adult life. Previously, we have successfully promoted adult neurogenesis and demonstrated functional recovery after hippocampal granule cell degeneration in a rat model. We used adrenalectomy (ADX) to induce gradual hippocampal granule cell death in a rat model. This model leads to a gradual loss of neurons, a feature more in line with neural

degenerative diseases. Treatment of ADX animals with sonic hedgehog (shh) can promote a significant amount of short term granule cell regeneration. However animals housed in enriched environment were capable of maintaining the survival of new neurons for longer term. The ability of the dentate gyrus to produce adult born neurons in both ADX and sham animals were examined. The number of new born neurons in the DG area of ADX animals was comparable to those observed in sham animals. These results suggest that environmental enrichment is critical for the survival of new born neurons in this experimental paradigm. Animals kept in regular cages without environmental enrichment were capable of producing adult born neurons, but the new born neurons cannot survival for long term(months).

Disclosures: **P. Wu:** None. **H. Lin:** None. **G. Lai:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.12/A12

Topic: A.02. Postnatal Neurogenesis

Support: Drottnings Silvias Jubileumsfond

Wilhelm och Martina Lundgren

Fru Mary Von Sydow

VR Grant 2014-3224

ALF Grant ALFGBG-450961

Hjärnfonden FO2014-0184

Title: Impact of muscular overexpression of PGC-1α on photothrombotic stroke in the mouse neocortex

Authors: *L. KARLSSON[1], N. GONZÁLEZ-ALVARADO[1], A. OSMAN[2], K. BLOMGREN[2], G. KUHN[1];
[1]Inst. for Neurosci. and Physiol., Univ. of Gothenburg, Gothenburg, Sweden; [2]Dept. of Women's and Children's Hlth., Karolinska Insitute, Stockholm, Sweden

Abstract: OBJECTIVE: To determine if overexpression of the exercise-induced transcription factor PGC-1α in skeletal muscle improves morphological stroke outcomes. METHODS: Male transgenic mice overexpressing PGC-1α under the muscle creatinine kinase (MCK) promoter were subjected to a photothrombotic stroke in the sensory-motor cortex area. Ischemic lesions were induced using Bengal rose and an infrared laser beam aimed at the exposed skull of 3-

month-old mice. Animals were perfused 4 weeks post-lesion for morphological analysis of lesion size, inflammatory response and neural progenitor migration. RESULTS: Muscular overexpression of PGC-1α resulted in increased infarct size (0.97 ± 0.07 mm3, n=5) compared to wild type controls (0.58 ± 0.10 mm3, n=7; t-test, p=0.017). No differences could be detected in the peri-lesional size, defined as an area with a distinct reduction in neuronal density. We measured the inflammatory response by quantifying microglial and astrocytic markers. Total and activated numbers of microglial cells (IBA1/CD68) within the peri-lesional area were similar for the transgenic and wild type groups. As a measure of reactive astrocytosis, the radial intensity profile of the astrocytic marker (GFAP) was quantified up to a distance of 1.6 mm from the infarct border. We were unable to detect any differences in the intensity profile between the two groups in neither stroke nor control conditions. Furthermore, no difference could be detected in the migration of neural progenitor cells (DCX) from the sub-ventricular zone to the lesioned neocortex. CONCLUSION: Contrary to our expectations, we found that overexpression of PGC-1α does not seem to have beneficial effects on morphological stroke outcomes in mice, but rather displays detrimental effects, resulting in increased infarct size. This suggests that muscular expression of PGC-1α has an impact on recovery and rescue of penumbral tissue through mechanisms that need to be analyzed in further studies.

Disclosures: L. Karlsson: None. **N. González-Alvarado:** None. **A. Osman:** None. **K. Blomgren:** None. **G. Kuhn:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.13/A13

Topic: A.02. Postnatal Neurogenesis

Support: PhD Scholarship: DAAD / CNPq

Title: Effects of reduced adult neurogenesis on cognition and emotional behavior of mice and environmental strategies as possible rescue

Authors: *M. E. SAKALEM[1], T. SEIDENBECHER[3], R. SAFFARI[2], M. ZHANG[2], K. DIEDERICH[4], J. C. SCHWAMBORN[5], W. ZHANG[2], O. AMBRÉE[2];
[2]Dept. of Psychiatry and Psychotherapy, [1]Universitätsklinikum Münster, Münster, Germany; [3]Inst. of Physiol. I, [4]Dept. of Neurol., Univ. Münster, Münster, Germany; [5]Fakultät für Naturwissenschaften, Technologie und Kommunikation, Univ. Luxemburg, Luxemburg, Luxembourg

Abstract: Although it is now accepted that newborn neural cells are continuously integrated into distinct areas of the adult mammalian brain throughout life (a process referred to as adult

neurogenesis), its functional role remains rather unclear. Much has been speculated and investigated; nevertheless there is controversy on how adult neurogenesis contributes to memory processes and emotional behavior. The aim of this study was to investigate the influence of adult newborn neural progenitors on anxiety-related behavior as well as recognition and emotional memory. For this purpose, dividing progenitor cells were depleted by ganciclovir treatment in GFAP-tk transgenic mice. After the pharmacological depletion, a two-week rest was presented prior to behavioral testing in order to allow the new differentiating neurons to be functional and start integrating into the neural circuitry. The behavioral tests used were Open Field, Object Recognition and Contextual Fear Conditioning. After testing, the brains were collected for immunohistochemistry. BrdU and Doublecortin stainings confirmed a clear reduction in the number of proliferating neural progenitor cells in transgenic mice in comparison with wild types, as well as in the number of immature neurons, respectively. With regard to the behavior, transgenic animals presented no deficit in general locomotion; also no anxiety-related changes were observed. Nevertheless, recognition memory was evidently impaired in the animals with reduced neurogenesis, in addition to an increased freezing response in the Fear Conditioning Test. In order to investigate a possible rescue effect on these transgenic mice, a combination of two neurogenesis-stimulant factors is currently carried out. For this study, after the pharmacological depletion, animals undergo a 3-week protocol with enriched environment combined with physical exercise in running wheels, for 1 hour a day. By manipulating the environment this way, it is expected that not only the remaining neural progenitor cells will be stimulated to proliferate, but their survival rate will also be supported. The results of this manipulation on recognition and emotional memory as well as adult neurogenesis will be presented.

Disclosures: **M.E. Sakalem:** None. **T. Seidenbecher:** None. **R. Saffari:** None. **M. Zhang:** None. **K. Diederich:** None. **J.C. Schwamborn:** None. **W. Zhang:** None. **O. Ambrée:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.14/A14

Topic: A.02. Postnatal Neurogenesis

Support: MH093650

MH091945

DA030425

Title: Extended intermittent access to palatable food decreases hippocampal neurogenesis and impairs hippocampal function

Authors: *A. FERRAGUD, C. VELÁZQUEZ-SÁNCHEZ, A. AL ABDULLATIF, V. SABINO, P. COTTONE;
Pharmacol. and Psychiatry, Boston Univ., Boston, MA

Abstract: Development of compulsive eating in eating disorders and obesity is promoted by cycles of access to and deprivation from highly palatable food, and resembles that of addiction to drugs. Epidemiological studies have shown that forms of compulsive eating in obesity and eating disorders are also accompanied by cognitive deficits, which have been proposed to result from a diet-induced reduction in hippocampal plasticity. Here, we studied the effects of intermittent access to palatable food on hippocampal neurogenesis, a pivotal mechanism of plasticity, and on hippocampal function. Male Wistar rats were either fed chow continuously for 7 days/week (Chow/Chow group), or fed chow intermittently 5 days/week, followed by a high-sucrose, palatable diet 2 days/week (Chow/Palatable group). Following diet cycling, hippocampal function was assessed either during acute withdrawal or following renewed access to palatable food. Our results show that animals withdrawn from palatable food displayed deficits in the ability to recognize a novel location of a familiar object, as well as a bias in their preference of a "novel cue" over a "novel place", compared to Chow/Chow animals. Furthermore, diet cycled rats showed reduced expression of immature neurons in the dentate gyrus of the hippocampus as well as a withdrawal-dependent decrease of proliferating cells. In summary, our results suggest that intermittent access to highly palatable food impairs hippocampal function and reduces hippocampal neurogenesis.

Disclosures: A. Ferragud: None. **C. Velázquez-Sánchez:** None. **A. Al Abdullatif:** None. **V. Sabino:** None. **P. Cottone:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.15/A15

Topic: A.02. Postnatal Neurogenesis

Support: NSF IOS 0818259

NSF IOS 1121345

Title: Cytokine regulation of adult neurogenesis: The immune system provides neuronal precursors for adult neurogenesis

Authors: *B. S. BELTZ, E. COCKEY, J. LI, J. L. BENTON;
Neurosci. Program, Wellesley Col., Wellesley, MA

Abstract: Adult neurogenesis, the creation of new neurons in the adult brain, is an evolutionarily conserved process that occurs in both vertebrate and invertebrate species. In the crayfish Procambarus clarkii, the 1st-generation neuronal precursors are not self-renewing, however the pool of neuronal precursors in the niche is never depleted over the long lives of these animals. Thus, neuronal precursors in the crayfish must be added to the neurogenic niche from an extrinsic source. *In vitro* and *in vivo* findings have shown that hemocytes generated by the immune system are the likely neuronal precursors. In the current studies, three protocols are used to extend these findings and to further characterize the relationship between the immune system and adult neurogenesis in P. clarkii. Cells labeled with 5-bromo-2'-deoxyuridine (BrdU) were quantified in the neurogenic niche and immune system tissues (the anterior proliferation center [APC] and posterior hematopoietic tissue [HPT]) at standardized time points over a 21 day period. The dynamics within the immune system were then altered with recombinant astakine 1 (r-AST1), a cytokine in the prokineticin family that promotes the release of hemocytes into the circulation. The effects on precursors in the neurogenic niche were determined. The resulting data show that manipulating the immune system of P. clarkii with r-AST1 alters the timeline by which neuronal precursors arrive in the neurogenic niche, confirming that neuronal precursors in the crayfish are indeed derived from the innate immune system.

Disclosures: **B.S. Beltz:** None. **E. Cockey:** None. **J. Li:** None. **J.L. Benton:** None.

Poster

289. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 289.16/A16

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant EY012245

Title: Bipolar cell death precedes retinal ganglion neuron loss in a complex 1 deficiency mouse model

Authors: *L. SONG;
Univ. of California-Davis, Davis, CA

Abstract: Mitochondrial complex 1 deficiency is associated with a wide spectrum of neurodegenerative diseases such as Leber hereditary optic neuropathy and Parkinson's disease. The molecular basis of neuronal death mediated by defective complex 1 activity remains unclear. Ndufs4 is an important subunit of complex 1, and its loss causes complex 1 deficiency. Ndufs4-/- mice have acute vision loss between p20 and p30 days. Here we explore the retinal pathology occurring between p20 and p30 in the Ndufs4-/- mice. Our results showed that Ndufs4-/- retinas exhibited significant reduction in bipolar cells and postsynaptic density protein 95 accompanied

by retinal ganglion cell dendritic atrophy, but retinal ganglion cell counts were similar at this point between Ndufs4-/- and wildtype mice. Our previous published data indicated that there is loss of retinal ganglion cells in retinas of Ndufs4-/- mice at around p42 days. In addition, we found that apoptosis was significantly increased in optic nerve, inner and outer nuclear layer of Ndufs4 -/- mice at p20-21 days, and this result was confirmed by Western blot and QRT-PCR. Ndufs4-/- retinas also exhibited increased oxidative stress as manifested by increased 4HNE, HO1 and Cuzn-SOD at p20 days. Our results cast light onto the mechanism of retinal ganglion cell death induced by complex 1 deficiency and may have implications for the development of new therapies and early detection for blinding mitochondrial disease.

Disclosures: L. Song: None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.01/A17

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: SDSU UGP 242499 to HSB

SDSU Undergraduate Research Grant to CF & HSB

SDSU Undergraduate Research Grant to CF, SMS & HSB

Title: Maturation of postural sway is not influenced by body mass index or gender

Authors: *C. FRENCHIK, S. M. SORIA, S. SCHULMAN, D. PICARDI, D. J. GOBLE, H. S. BAWEJA;
San Diego State Univ., San Diego, CA

Abstract: The purpose of this study was to determine the age by which children attain and exhibit adult like postural sway characteristics during quiet unperturbed standing. In addition, we examined whether development of postural sway control was influenced by gender and body mass index (BMI). 50 children between the ages of 7-12 years (19 females), 39 children between the ages of 13-17 (17 females) and 70 young adults between the ages of 18-24 years (30 females) volunteered to participate in the study. The Balance Tracking System (BTrackS, San Diego, CA) force plate was used to assess the postural sway during quiet standing for all trial. Testing consisted of 3 trials of quiet standing with eyes closed with feet shoulder width apart and hands on the hips. Each trial lasted 20 seconds during which the total center of pressure (COP) sway, COP antero-posterior sway, COP medio-lateral sway path-lengths were calculated. A principle component analysis was used to calculate the 95 and 99% confidence intervals of the area within which the COP would lie. Overall, Children from 7-12 years of age exhibited greater postural

sway when compared with 13-17 year olds and young adults. However, 13-17 year old children exhibited similar postural sway characteristics to those of young adults. There were no differences in postural sway between males and females irrespective of the age and BMI. As expected the BMI increased significantly with age, but only accounted for ~ 18% of the variability in total COP sway path-length. Our tests relied heavily on vestibular and proprioceptive integration as they were performed only with eyes-closed. Therefore, our findings are indicative of an inefficient multisensory integration in children up to the age of 12 years. Our findings support and extend previous findings suggesting that children do not exhibit adult-like sensory integration prior to the age of 12 years.

Disclosures: C. Frenchik: None. **S.M. Soria:** None. **S. Schulman:** None. **D. Picardi:** None. **D.J. Goble:** None. **H.S. Baweja:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.02/A18

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NIDRR H133E10007

AACPDM Research Grant

Title: Changes in ankle joint proprioception resulting from robotic training of ankle mobility rehabilitation in cerebral palsy children

Authors: *Y. LEE, D. XU, Y. REN, K. CHEN, D. GAEBLER-SPIRA, L.-Q. ZHANG; Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Cerebral palsy (CP) is the leading cause of childhood motor disability. Although brain lesions in CP are not progressive, children with CP continue to experience the motor difficulties caused by the secondary impairments, such as spasticity, muscle weakness, reduced selective motor control and sensory deficits. Furthermore, although sensory function plays an important role in functional activities, sensory deficit has not been well studied in the children with CP. The objectives of this study were to 1) investigate proprioception deficit at the ankle in CP; 2) improvement in proprioception through robotic ankle stretching and active movement training. Seven children with CP participated in the combined passive stretching and active movement training using an ankle rehabilitation robot. Eight healthy children were recruited for one-time assessment of ankle proprioception. Children with CP received robotic training 45 minutes/session, 3 sessions/week for a total of 18 sessions. Each session consisted of 10 min intelligent stretching followed by 20 min active movement training and ended with 10 min stretching. Proprioception was measured as the plantar or dorsi-flexion movement (at 0.5°/sec

controlled by the ankle rehabilitation robot) when the children felt the movement. The evaluation was done at two time points: pre-treatment and post-treatment. First, children with CP had impaired proprioception. Their ankle proprioception acuity was 3.90±2.40° (mean±SD) in dorsiflexion and from 3.96±2.46° in plantar flexion, as compared to that of the healthy controls' 0.94±0.43° in dorsiflexion ($p<0.01$) and 0.86±0.48° in plantar flexion ($p<0.01$). Furthermore, children with CP sometimes detected the movement in wrong direction. For children with CP gone through multiple sessions of the robot-guided therapy, the results showed an ankle sensory improvement after the robotic training. The proprioception angle threshold reduced from 3.90±2.40° to 3.28±2.15° in dorsiflexion ($p=0.01$) and from 3.96±2.46° to 2.72±2.05° in plantar flexion ($p=0.18$). Result suggests ankle proprioception deficit in CP. Furthermore, the use of robotic training for CP children reduced the ankle sensory deficit, suggesting sensory impairment in CP can be improved through controlled stretching and active movement training.

Disclosures: **Y. Lee:** None. **D. Xu:** None. **Y. Ren:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rehabtek LLC. **K. Chen:** None. **D. Gaebler-Spira:** None. **L. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rehabtek LLC.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.03/A19

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: R01-HD063071

R37- HD081168

Fulbright Graduate Student Program

Title: Twitches drive neural activity in the deep cerebellar nuclei of sleeping newborn rats: Implications for sensorimotor development

Authors: ***A. M. PLUMEAU**[1,2], C. DEL RIO-BERMUDEZ[1], G. SOKOLOFF[1], M. BLUMBERG[1,2];
[1]Psychological and Brain Sci., [2]Interdisciplinary Grad. Program in Neurosci., Univ. of Iowa, Iowa City, IA

Abstract: Spontaneous muscle twitches during active sleep drive robust neural responses in the developing brain that are thought to be critical for the development of sensorimotor systems. The cerebellum is one structure that likely serves as a comparator of sensory and motor signals. In

rats, the cerebellar circuit undergoes extensive postnatal reorganization, with many transient synapses appearing and disappearing over the first few postnatal weeks. Recent work from our lab has shown that twitching drives activity in the cerebellar cortex during this period. However, little is known about twitch-related activity in the deep cerebellar nuclei, the primary output of the cerebellum. In this study, we recorded multiunit activity and local field potentials from the anterior interpositus and dentate nuclei of unanesthetized, headfixed 8- and 12-day-old rats as they cycled between sleep and wake. Forelimb, hindlimb, and nuchal EMGs were also recorded and an experimenter simultaneously scored behavior. The majority of neurons from both the interpositus and the dentate showed increased activity approximately 70 ms after twitches of the forelimb or hindlimb. Additionally, we observed rhythmic unit firing at both ages at a frequency of 8-10 Hz. Finally, at 12 days of age some cells exhibited a slow activity rhythm of 3-5 Hz that occurred during behavioral quiescence. Together with recent findings of twitch-related activity in other associated structures_including the red nucleus and inferior olive_these results provide further support for the hypothesis that twitching, as a substantial contributor to neural activity in the infant brain, promotes plasticity and somatotopy in the developing sensorimotor system.

Disclosures: A.M. Plumeau: None. **C. Del Rio-Bermudez:** None. **G. Sokoloff:** None. **M. Blumberg:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.04/A20

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: R01-HD063071

R37- HD081168

Title: The inferior olive processes twitch-related information during active sleep in newborn rats: evidence of corollary discharge

Authors: *D. MUKHERJEE, G. SOKOLOFF, M. S. BLUMBERG;
Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: The inferior olivary nucleus (IO) plays an important role in modulating motor activity in mammalian species, including rats. Located within the brainstem, climbing fibers from the IO project to Purkinje cells in the cerebellar cortex where they trigger complex spikes. Various functions for IO activity have been proposed. For example, the IO is thought to provide teaching signals for the execution of skilled motor movements or error signals regarding inappropriately performed movements. The applicability of such notions to the early expression of IO activity is not known. We have shown that during the first two postnatal weeks in rats, active (or REM)

sleep-related twitches trigger complex spikes, suggesting a role for twitching in activity-dependent development of cerebellar Purkinje cells. Based on these findings, we hypothesized that the IO would exhibit twitch-related activity. To test this hypothesis, we recorded extracellular multiunit activity and local field potentials from the IO of head-fixed unanesthetized 8- and 12-day-old rats that were cycling normally between sleep and wake. IO neurons exhibited phasic activity that was particularly pronounced immediately after twitches of the nuchal and forelimb muscles. This twitch-related IO activity was remarkably precise and pronounced, reaching a peak in firing rate within 0-10 ms after a twitch. Moreover, some IO neurons exhibited rhythmic firing with a frequency of 8-10 Hz. Importantly, IO neurons exhibited little or no activity during wake movements or in response to exaffarent stimulation of the limbs. Also, local field potentials exhibited a prominent 2-Hz rhythm primarily during periods of quiescence, likely reflecting subthreshold oscillations of IO neurons as has been reported in adults. These findings confirm that twitch-related information is processed by the IO during early infancy. Moreover, the timing of IO activation suggests that this nucleus is receiving a motor copy or corollary discharge signal, perhaps from the red nucleus that is known to contribute to the production of twitches. Processing of this information by the IO during development could play an important role in motor learning and sensorimotor integration.

Disclosures: D. Mukherjee: None. **G. Sokoloff:** None. **M.S. Blumberg:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.05/A21

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: R37-HD081168

R01-HD063071

Fulbright Foreign Student Program

Title: Sensorimotor integration in the red nucleus of infant rats during active sleep

Authors: *C. DEL RIO BERMUDEZ, G. SOKOLOFF, M. BLUMBERG;
Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: Sensorimotor integration of self-generated movements and their associated sensory feedback (i.e., reafference) is necessary for the early development of somatotopic maps and coordinated motor skills. During infancy, self-generated movements are not restricted to periods of wakefulness. On the contrary, sensory feedback from myoclonic twitching during active (or REM) sleep is thought to drive activity-dependent development of the perinatal nervous system.

We hypothesized that the red nucleus (RN), source of the rubrospinal tract (RST), is not only a major source of motor output for twitching early in development, but also processes twitch-related reafference in a somatotopic fashion. We recorded extracellular neural activity in the RN during sleep and wakefulness in 8- and 12-day-old unanesthetized rats. Neuronal activity in the RN significantly increased before twitching of the contralateral forelimb, consistent with a role of the RN in the production of twitches. In fact, unilateral inactivation of the RN using a cocktail comprising GABAA, GABAB, and glycine receptor agonists markedly decreased twitching. In addition, a subpopulation of neurons exhibited significant increases in activity after twitches, suggesting reafferent sensory processing. Analysis of local field potentials and multiunit activity in the RN revealed distinct state-dependent and age-related patterns of activity. Overall firing rates increased substantially by the end of the second postnatal week as rhythmic activity patterns emerged. These developmental changes in firing properties of RN neurons suggest the emergence of new functional connections with other related structures, such as the deep cerebellar nuclei. We propose that twitches, which are characterized by discrete motor output and reafferent input, provide an opportunity for the activity-dependent development of sensorimotor integration and somatotopy within the newborn RN and associated neural networks.

Disclosures: C. Del Rio Bermudez: None. **G. Sokoloff:** None. **M. Blumberg:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.06/A22

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NIH grant DC011534 to ASL

NIH grant DE022065 to SAM

NIH grant HD083157 to TMM, SAM and ASL

Title: Mosaic regulation of placodal and neural crest-derived cranial sensory neurons establishes distinct developmental states during early neuronal differentiation and axon outgrowth

Authors: B. A. KARPINSKI[1,2], C. BRYAN[1,3], A. HORVATH[1,4], A. FERNANDEZ[1,3], J. L. BAKER[1,5], T. M. MAYNARD[1,3], S. A. MOODY[1,2], *A.-S. LA MANTIA[1,3];
[1]Inst. for Neurosci., [2]Dept. of Anat. & Regenerative Biol., [3]Dept. of Pharmacol. and Physiol., [4]McCormick Genomic and Proteomic Ctr., The George Washington Univ. Sch. of Med., Washington, DC; [5]Ctr. for the Advanced Study of Human Paleobiology, The George Washington Univ., Washington, DC

Abstract: We assessed transcriptional, signaling, and cellular mechanisms that regulate initial differentiation of placodal and neural crest-derived cranial sensory neurons. We asked whether quantitative expression of 21 transcription factors associated with these cells defined an anterior-posterior gradient of sensory neuron identity. With few exceptions we found that a mosaic of transcription factors, rather than consistent gradients, identifies cranial sensory neurons or precursors in the olfactory placode, trigeminal ganglion, geniculate/vestibular/acoustic ganglia, otic placode, and petrosal/nodose ganglia. There is a similar quantitative mosaic of cell lineages for placodal or neural crest-derived sensory neurons. Disrupting the activity of two signaling pathways, via Fgfs or RA, believed to be essential determinants of cranial A-P identity modified this transcriptional and cellular mosaic; however, these changes did not follow an A-P gradient. Instead, expression levels of key factors and proportions of distinct lineage cohorts are locally sensitive to diminished Fgf and RA signaling. The primary consequence of disrupting Fgf signaling was to shift proportions of placodal and neural crest-derived populations and in parallel arrest cranial sensory axon outgrowth. The primary consequence of disrupting RA signaling was to alter axon trajectories of cranial sensory neurons. In each instance, local changes of transcription factor expression accompanied locally distinct altered sensory axon growth. Together our data show that there is not a graded transcriptional code in the A-P axis for cranial sensory neuron differentiation. Instead, there is a transcriptional mosaic, locally modulated by morphogenetic signals, that parallels lineage contributions from cranial placodes and neural crest. This mosaic is associated with the developmental state of diverse cranial sensory neurons, likely insuring appropriate axon growth and targeting.

Disclosures: **B.A. Karpinski:** None. **C. Bryan:** None. **A. Horvath:** None. **A. Fernandez:** None. **J.L. Baker:** None. **T.M. Maynard:** None. **S.A. Moody:** None. **A. La Mantia:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.07/A23

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: CONACYT Grant 181334 G3 (IJE)

Title: Electrophysiological recording of the sensory sural nerves from artificial reared pups of 14 and 21 postnatal days: Role of tactile stimulation

Authors: ***A. I. MELO**[1], S. MORENO-PÉREZ[2], G. RAMÍREZ-FUNEZ[4], I. JIMÉNEZ-ESTRADA[5], M. E. MENDOZA -GARRIDO[5], B. SEGURA[6], M. GONZÁLEZ DEL PLIEGO[7], E. L. AGUIRRE-BENITEZ[7], J. HERNÁNDEZ-FALCÓN[8], A. S. FLEMING[9], R. ZEMPOALTECA-RAMÍREZ[3];
[1]Cinvestav-lab.tlax.Universidad Autónoma De Tlaxcala, Tlaxcala, Mexico; [2]Agrobiología, Lic.

Biología, [3]CTBC, Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; [4]Escuela de Biología, BUAP, Puebla, Mexico; [5]Physiology, Biophysics and Neurosci., CINVESTAV-IPN, México, Mexico; [6]Biol., UNAM, FES Iztacala, México, Mexico; [7]Embriología, Medicina, [8]Lab. Redes Neuronales, Fisiología, Medicina, UNAM, México, Mexico; [9]Psychology, Univ. of Toronto, Mississauga, ON, Canada

Abstract: We recently found that the area of the compound action potential (CAP) evoked in sensory sural nerves (SU n.) of adult male rats that were total maternal deprived and artificial reared (AR) was smaller than the CAP provoked in SU n. from mother reared (MR) and AR social rats (reared with two same-age foster pups). In order to determinate at what age such effects appears, during isolation, male pups rats of 4 postnatal days (PND) were reared artificially (AR group) until 14 or 21 PND. At such ages, the electrophysiological properties of the SU n. were recorded. As control groups, we recorded the CAP of SU n. from pups reared with their mother (MR group), or that were AR but received five bouts of perineal+body tactile stimulation a day (AR-Tactile group) with a paint brush. We found that the electrical threshold necessary to generate the CAP of SU n. from AR pups at 14, but not at 21PND, was significant higher than that determined for SU n. from MR pups ($p<0.05$, all comparisons). Furthermore, the amplitude and area of the maximal CAP response provoked in SU n. from AR pups with 14 and 21PND were significant shorter than that of SU n. from MR pups ($p<0.05$, all comparisons). The conduction velocity of the CAP was similar among groups. The replacement of the tactile stimuli during isolation prevented most of the effects, i.e., the CAP evoked in SU n. from AR-Tactile pups showed no significant differences with respect to that of MR nerves. These results suggest that AR provokes disturbances in the electrophysiological properties of SU nerves at early stages of postnatal development (14 and 21 PND) which can be prevented by perineal+body tactile stimulation.

Disclosures: **A.I. Melo:** None. **S. Moreno-Pérez:** None. **G. Ramírez-Funez:** None. **I. Jiménez-Estrada:** None. **M.E. Mendoza -Garrido:** None. **B. Segura:** None. **M. González del Pliego:** None. **E.L. Aguirre-Benitez:** None. **J. Hernández-Falcón:** None. **A.S. Fleming:** None. **R. Zempoalteca-Ramírez:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.08/A24

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: Sara and Frank McKnight Fellow

Title: Elucidating the role of spinal cord Atoh1-lineage neurons in proprioception

Authors: *H. C. LAI;
UT Southwestern Med., Dallas, TX

Abstract: ATOH1, a basic helix-loop-helix (bHLH) transcription factor, is transiently expressed in the dorsal-most part of the developing neural tube. Atoh1-lineage neurons in the spinal cord have been implicated in forming cerebellar-projecting neurons of the dorsal spinocerebellar tract (DSCT); however, it is unknown precisely how they contribute to the DSCT. We find that Atoh1-lineage neurons reside outside Clarke's column, a major contributor of neurons to the DSCT. In addition, elimination of caudal Atoh1-lineage neurons results in mice that are unable to perform coordinated motor tasks. Altogether, our findings suggest that DSCT neurons derive from more than one developmental source, which can be used to distinguish subsets of the proprioceptive system.

Disclosures: H.C. Lai: None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.09/A25

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant R01 EY022987

NIH Grant T32-EY015387-05

Title: Changes in thalamic connectivity of primary somatosensory cortex resulting from early bilateral enucleations in the short-tailed opossum (Monodelphis domestica)

Authors: *J. C. DOOLEY[1], M. S. DONALDSON[2], L. A. KRUBITZER[2];
[1]Ctr. for Neurosci., UC Davis, Davis, CA; [2]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Previously, our laboratory has examined the effects bilateral enucleations that occur at extremely early stages of development, before the arrival of retinal ganglion cell axons to their targets. Because opossums are altricial at birth, enucleations at very early stages of development can be made ex utero, at postnatal day 4 (P4). We demonstrated that neurons in the caudal pole of cortex (primary visual cortex in sighted animals) become responsive to somatosensory and auditory stimuli following early visual loss. These results were corroborated by connectional studies, indicating that large-scale changes in thalamocortical as well as cortico-cortical connectivity were also observed for cortex that would normally develop into V1. Additionally, we have shown that early loss of vision also affects the connectivity of the spared sensory systems, with numerous exuberant cortical projections to primary somatosensory cortex (S1).

However, whether this very early visual loss alters thalamic projections to S1 has yet to be determined. In these studies we utilized neuroanatomical tracing techniques to examine thalamocortical connections of S1 in opossums that were bilaterally enucleated at P4, before thalamocortical connections innervate the neocortex. Following injections of anatomical tracers in S1, we quantified the proportion of labeled neurons in different, architectonically defined nuclei of the thalamus. Unlike cortical connections of S1 in bilaterally enucleated opossums, thalamic projections to S1 maintained the same modality-specific pattern of connectivity to S1 as in normal animals. However, there were differences in the composition of thalamic projections to S1 in enucleates, with fewer neurons (as a percent of the total number of labeled neurons in the thalamus) originating from the ventral posterior nucleus, and a greater number originating from both the posterior and ventral medial nuclei. These results suggest that while early enucleation does not induce aberrant projections to S1 from nuclei traditionally associated with the visual and auditory processing (e.g. MG, LGN), differences in the thalamic connectivity of S1 are still present in these animals suggesting that cross-modal plasticity is restricted to cortico-cortical connections of S1.

Disclosures: J.C. Dooley: None. **M.S. Donaldson:** None. **L.A. Krubitzer:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.10/A26

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Title: PRDM12 is a novel, evolutionarily conserved transcription factor that controls sensory neuron specification and nociception

Authors: *V. NAGY[1], T. COLE[2,3], C. VAN CAMPENHOUT[4], T. M. KHOUNG[2,3], C. LEUNG[2,5], S. VERMEIREN[4], M. NOVATCHKOVA[1], D. WENZEL[1], D. CIKES[1], A. A. POLYANSKY[6], I. KOZIERADZKI[1], A. MEIXNER[1], E. J. BELLEFROID[4], G. G. NEELY[2,3], J. M. PENNINGER[1];
[1]Inst. of Mol. Biotech., Vienna, Austria; [2]Garvan Inst. of Med. Res., Darlinghurst, Australia; [3]Charles Perkins Ctr. and Sch. of Mol. Biosci., Sydney, Australia; [4]Univ. Libre de Bruxelles, Gosselies, Belgium; [5]UNSW Med., Sydney, Australia; [6]Max Perutz Labs., Vienna, Austria

Abstract: PR homology domain-containing member 12 (PRDM12) is a transcription factor belonging to a conserved family implicated in cell fate decisions. PRDM12 plays an important role in the development of the neural crest in several species and plays a potential role in pathogenesis of chronic myeloid leukaemia in humans. PRDM12 is almost exclusively expressed in the central and peripheral nervous system, however, very little is known of its downstream targets or the potential role it might play in neuronal cell fate decisions and functions. We found

that PRDM12 is a key regulator of sensory neuronal specification in Xenopus. Further, we modeled PRDM12 mutations that cause hereditary sensory and autonomic neuropathy (HSAN) and show remarkable conservation of the mutated residues in evolution. Expression of wild-type human PRDM12 in Xenopus induced the expression of sensory neuronal markers, which were reduced using various disease-causing human PRDM12 mutants. In *Drosophila*, we identified Hamlet as the functional PRDM12 homologue that controls nociceptive behavior in sensory neurons. Furthermore, expression analysis of human patient fibroblasts with PRDM12 mutations uncovered possible downstream target genes. Knockdown of several of these target genes including thyrotropin-releasing hormone degrading enzyme (TRHDE) in *Drosophila* sensory neurons resulted in altered cellular morphology and impaired nociception. These data show that PRDM12 and its functional fly homologue Hamlet are evolutionary conserved master regulators of sensory neuronal specification and play a critical role in pain perception. Our data also uncover novel pathways in multiple species that regulate evolutionary conserved nociception. In addition, we are investigating the role PRDM12 and its downstream targets plays in development and sensory neuronal function in conditional mutant mouse models.

Disclosures: **V. Nagy:** None. **T. Cole:** None. **C. Van Campenhout:** None. **T.M. Khoung:** None. **C. Leung:** None. **S. Vermeiren:** None. **M. Novatchkova:** None. **D. Wenzel:** None. **D. Cikes:** None. **A.A. Polyansky:** None. **I. Kozieradzki:** None. **A. Meixner:** None. **E.J. Bellefroid:** None. **G.G. Neely:** None. **J.M. Penninger:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.11/A27

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: GACR P303/12/1347

Title: A detailed Golgi-Cox morphological analysis of the auditory cortex and inferior colliculus in rats exposed to an acoustically enriched environment

Authors: ***J. BURIANOVA**, L. OUDA, J. SYKA;
Inst. of Exptl. Medicine, CAS CZ, Prague, Czech Republic

Abstract: Evidence has accumulated that various kinds of acoustic exposures during the critical period of development can have either a positive or negative impact on the morphology of the central auditory system. In our previous study (Ouda et al., 2014), we demonstrated that early postnatal short noise exposure (8 min, 125 dB, day 14) resulted in the remodeling of neuronal trees in the central auditory system, comprising of a longer mean total length of neuronal trees in the inferior colliculus (IC) and medial geniculate body (MGB), a shorter mean total length of

apical dendrites in pyramidal cortical neurons of the auditory cortex (AC), and a higher numerical density of spines on branches of the pyramidal AC neurons. In the present study, we analyzed, using the Golgi-Cox method, the morphology of neurons in the IC and AC of 4-month-old Long-Evans rats exposed to an acoustically enriched environment (AEE) during the third and fourth weeks of life. In AEE exposed rats, the mean total length of the neuronal tree was found to be greater than in control animals in the external cortex and the central nucleus of the IC. In the AC, the mean total length of the basal dendritic segments of pyramidal neurons was significantly longer in AEE exposed rats, however, only slight differences with respect to controls were observed in the length of apical dendrites of pyramidal cells. These findings demonstrate that early AEE exposure can induce permanent changes in the development of neurons in the central auditory system, which apparently represent morphological correlates of functional plasticity.

Disclosures: **J. Burianova:** None. **L. Ouda:** None. **J. Syka:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.12/A28

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant NS073869

Title: Lack of muscle spindles in infant ErbB2 knockout mice is associated with deficits in functional and anatomical cerebellar development

Authors: N. J. SATTLER[1], A. J. YONK[1], C. M. COLEMAN[1], *G. SOKOLOFF[2], M. S. BLUMBERG[1];
[1]Psychological and Brain Sci., [2]Psychology, The Univ. of Iowa, Iowa City, IA

Abstract: The cerebellum, which exhibits protracted postnatal development, is important for sensorimotor integration. Previous studies in infant rats have shown that sensory feedback from myoclonic twitches, which occur exclusively during active (or REM) sleep, is a substantial driver of Purkinje cell activity. To further examine the contributions of proprioceptive feedback to twitch-related cerebellar activity and assess its role in cerebellar development, we investigated the neurophysiology, anatomy, and behavior of ErbB2 conditional knockout mice_which fail to develop functional muscle spindles_over the first two postnatal weeks. Because both climbing fibers and mossy fibers convey proprioceptive information to Purkinje cells, we hypothesized that knockout mice would exhibit fewer complex and simple spikes than wild-type mice. We further hypothesized that knockout mice would exhibit twitch-dependent Purkinje cell activity. Next, we assessed climbing fiber innervation of Purkinje cells as evidenced by the distribution of the vesicular glutamate 2 transporter (VGLUT2). Finally, we tested knockout and wild-type mice

on a cerebellar-dependent balance beam task. At 8 days of age, knockout mice exhibited reduced Purkinje cell activity, weaker twitch-related neural activity, and altered neural rhythmicity. VGLUT2 labeling differed between knockouts and wild types with respect to translocation of climbing fibers as well as distribution of vesicles across the Purkinje cell and molecular layers. At 15 days of age, knockout mice exhibited pronounced deficits on the balance beam task; at narrow widths, footslips increased significantly and latencies to traverse the beam increased. Taken together, these results support the hypothesis that proprioceptive input from muscle spindles contributes substantially to the functional and anatomical development of the cerebellum.

Disclosures: N.J. Sattler: None. **A.J. Yonk:** None. **C.M. Coleman:** None. **G. Sokoloff:** None. **M.S. Blumberg:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.13/A29

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Title: Highly patterned spontaneous activity in the developing mammalian auditory system *in vivo*

Authors: *A. P. LOMBROSO[1], A. GRIBIZIS[1], H. C. WANG[2], W. ZHANG[2], T. BABOLA[2], D. BERGLES[2,3], M. CRAIR[1];
[1]Dept. of Neurobio., Yale Univ., New Haven, CT; [2]Solomon H. Snyder Dept. of Neurosci., [3]Dept. of Otolaryngology Head and Neck Surgery, Johns Hopkins Univ., Baltimore, MD

Abstract: Spontaneous activity is a common feature of developing sensory systems. In the visual system, waves of spontaneous activity generated in the retina propagate to the thalamus and cortex before the retina is visually responsive. This patterned spontaneous activity helps to define neuronal pathways in the ascending visual system. In the developing auditory system, bursts of activity initiated within the cochlea before hearing onset may play a similar role in refining auditory system neural circuits. However, relatively little is known about the spatial and temporal patterns of this spontaneous activity in the ascending mammalian auditory system, and its role in development remains to be defined. To examine developmental changes in this sensory-independent activity and determine the mechanisms responsible, we imaged global changes in neuronal activity in the developing inferior colliculus (IC) and auditory cortex (A1) in awake neonatal mice using genetically encoded calcium indicators (GECIs). The IC plays a central role in the ascending auditory system, receiving input from auditory brainstem nuclei and relaying this activity to the MGN and eventually A1. We observed bands of activity in neonatal IC that were highly correlated between hemispheres and with activity in the ipsilateral auditory

cortex. These activity bands were stationary (they did not propagate across the IC), their orientation was consistent with tonotopic bands previously observed using anatomic and functional (electrophysiological) techniques, and they depend on input from the cochlea. Spontaneous bands of activity become more frequent, more refined, and of shorter duration as animals aged. By examining the source of this spontaneous activity and changes in its spatial and temporal properties during development, we hope to understand the role this early activity plays in preparing the auditory system for the onset of hearing.

Disclosures: A.P. Lombroso: None. **A. Gribizis:** None. **H.C. Wang:** None. **W. Zhang:** None. **T. Babola:** None. **D. Bergles:** None. **M. Crair:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.14/A30

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NSERC #312015

NSERC #3595

Title: Anatomical study of transient commissural projections between the vestibular ganglia in neonatal opossums (Monodelphis domestica)

Authors: *F. LANTHIER, A. LALONDE-LARUE, T. CABANA, J.-F. PFLIEGER;
Sci. Biologiques, Univ. De Montréal, Montreal, QC, Canada

Abstract: The opossum is born in a very immature state. It nevertheless has to crawl, unaided by the mother, from the urogenital opening to a nipple where it attaches to pursue its development. Sensory information must guide the newborn to a nipple and trigger its attachment to it. In a previous study, the possible involvement of the vestibular system, which acts on spinal motor networks in response to gravitational cues, was investigated by anatomical tracing. This study showed the existence of vestibular afferents to the utricle and to the vestibular complex of the brainstem as well as of vestibulospinal projections in the newborn opossums. An unexpected finding was the presence of vestibular afferents crossing to the contralateral vestibular ganglion. To investigate the development of these projections, Texas Red conjugated Dextran-Amine crystals were placed in a longitudinal section in the brainstem midline, from the level of the trigeminal ganglia to the level of the obex, in *in vitro* preparations of the head taken from opossums aged postnatal day 5 (P5) to P15. A 4.5 h period was allowed for transport of the tracer, in the dark. The tissue was then fixed, cryosectioned and the slides were observed under a fluorescence microscope. Labeling of cells and nerve fibers was abundant in the brainstem at all ages studied. More specifically, labeled cells in the vestibular ganglion and labeled fibers which

entered or exited the ganglion were observed, bilaterally. To examine the temporal evolution of this commissural projection, the labeled cells in the vestibular ganglia were counted. The number of labelled cells per ganglion increased from about 16 at P5 to a maximum of around 43 at P11, and then decreased to around 7 by P15, the oldest age available. These results confirm the existence of commissural projections between the left and right vestibular ganglia in early postnatal opossums and show their subsequent decrease by the end of the second postnatal week. The period of increase of the commissural vestibular projections corresponds to a period just before neurons in the vestibular nuclei start to express c-Fos, a marker of neuronal activity, in response to vestibular stimulation. This raises the question of a link between the vestibular ganglia commissural projections and vestibular function in neonatal opossums. We must further investigate if commissural projections are functional and if they totally disappear past P15.

Disclosures: F. Lanthier: None. **A. Lalonde-Larue:** None. **T. Cabana:** None. **J. Pflieger:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.15/A31

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: National Institute of Deafness and Other Communicative Disorders NIH Grant R21DC009879

University of Miami, College of Arts and Sciences Gabelli Fellowship to Zhongmin Lu

Title: Alcohol-sensitive period during early octavolateral organ development in Zebrafish (Danio rerio)

Authors: *L. ZAMORA[1,2], K. C. MIGUEL[2], Z. LU[1,2,3];
[1]Biol., [2]Neurosci. Program, Univ. of Miami, Coral Gables, FL; [3]Intl. Ctr. for Marine Studies, Shanghai Ocean Univ., Shanghai, China

Abstract: It is well known that fetal alcohol exposure can cause Fetal Alcohol Spectrum Disorders (FASD), a completely preventable developmental disability that is characterized by permanent birth defects. However, it is unclear whether or not there is a specific time window during gestation that fetuses are most sensitive to alcohol exposure. In this study, we used the zebrafish (*Danio rerio*) as a model to determine the alcohol-sensitive period in the first 24 hours post fertilization (hpf). Zebrafish's octavolateral organs, including inner ears and lateral line neuromasts, function in hearing, balance, and hydrodynamic detection, respectively. *SeqET4* zebrafish that express green fluorescent protein in all sensory hair cells were treated in 2% alcohol for 5-hour time windows from 2-7, 7-12, 12-17, and 17-22 hpf. Octavolateral organs of

control and alcohol-exposed larvae were morphologically examined at 3, 5, and 7 days post fertilization (dpf). Using confocal and light microscopy, we found all alcohol-exposed larvae had significantly smaller otic vesicles and saccular otoliths at 3 dpf. Only alcohol-exposed larvae from 12-17 hpf had smaller otic vesicles at 5 dpf and smaller saccular otoliths at 7 dpf. Number of saccular hair cells, number of neuromasts, and hair cells per neuromast were also decreased only for alcohol-exposed larvae from 12-17 hpf. Auditory function was also assessed at 3 dpf by inner ear recordings of extracellular microphonic potential responses to 200-Hz stimulation. Hearing sensitivity was significantly diminished only for larvae exposed to alcohol from 7-12 and 12-17 hpf. To determine if fewer hours of alcohol exposure between 12-17 hpf also caused detrimental defects of octavolateral organ morphology and function, embryos were alcohol-exposed from 12-14 and 14-17 hpf. Significantly smaller otic vesicles and saccular otoliths were observed after 2 and 3 hours of alcohol exposure, similar to all 5-hour alcohol-exposed larvae. Fewer number of neuromasts were also found at 3 dpf, similar to larvae alcohol-treated from 12-17 hpf. Saccular otoliths were also smaller at 5 dpf for 2-hour alcohol-exposed larvae. Although, neither number of hair cells per neuromasts nor hearing sensitivity was significantly affected for larvae alcohol-treated for 2 or 3 hours. Our results show that 12-17 hpf is an alcohol-sensitive time window when morphology and function of octavolateral organs in zebrafish are most vulnerable to embryonic alcohol exposure. This study demonstrates that precise timing of maternal alcohol consumption during fetal development can cause specific FASD symptoms, such as hearing deficits.

Disclosures: **L. Zamora:** None. **K.C. Miguel:** None. **Z. Lu:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.16/A32

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Title: Brief developmental hearing loss leads to a deficit in inhibitory synaptic transmission in the adult auditory striatum

Authors: ***T. M. MOWERY**, V. KOTAK, D. SANES;
New York Univ., New York, NY

Abstract: Background Critical periods have been studied almost exclusively in the primary sensory pathways, including auditory cortex (Mowery et al. 2014). However, deprived animals often display behavioral deficits that are not solely attributable to sensory processing. Here, using a cortico-striatal brain slice preparation, we explored whether transient hearing loss affects synaptic inhibition recorded from regions of adult striatum that receives input from auditory cortex (ACx) . Methods Whole-cell voltage-clamp recordings were obtained from ACx-recipient

putative medium spiny neurons (MSN) in cortico-striatal brain slices from gerbils (Meriones unguiculatus) that experienced transient mild hearing loss. Earplugs were inserted bilaterally on postnatal day (P) 11 and removed on P35; thus the animals experienced hearing from P35 until the day of recording (P86). MSNs from earplugged animals (n=12) were compared to those recorded from age-matched controls (n= 15). Results In transiently earplugged animals, spontaneous postsynaptic inhibitory current (sIPSC) was significantly smaller [mean ± SEM: control; -25.1 ± 3.6 pA vs. transient earplug: -12.7 ± 1.0 pA; $p < .05$). The sIPSC time constant was also significantly longer [control: 22.2 ± 2.7 ms vs. transient earplug: 30.1 ± 3.3 ms, $p < .05$). Finally, the sIPSC rate was significantly lower [control: 15.2 ± 2.7 Hz vs. transient earplug 3.77 ± 1.3 Hz, $p < .01$]. Conclusion: These results suggest that the striatum, a major non-sensory region downstream from sensory cortex, is sensitive to a brief period of acoustic deprivation. Furthermore, these effects persisted until adulthood, and this has implications concerning recovery from hearing loss in children.

Disclosures: **T.M. Mowery:** None. **V. Kotak:** None. **D. Sanes:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.17/A33

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: Battaglia Endowment

NIH T32 EB009384

Title: Mapping of pain circuitry in early post-natal development using manganese-enhanced MRI

Authors: ***M. M. SPERRY**[1], B. M. KANDEL[1], K. E. BASS[2], S. R. DAS[1], P. S. DHILLON[1], J. C. GEE[1], G. A. BARR[2];
[1]Bioengineering, Univ. of Pennsylvania, Philadelphia, PA; [2]Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Approximately 400,000 human infants are admitted to the neonatal intensive care unit in the United States every year, and undergo 10-20 or more medically essential but potentially painful procedures each day, often without analgesics. We know that the number of painful procedures in the human neonate is related to poorer outcomes but not how these painful procedures change brain function. Thus we have virtually no detailed understanding of the mechanisms by which the brain is engaged during pain early in life. Based on similarities in brain development between rat and human neonates, the goal of this study was to use MEMRI to map brain circuits that are activated following peripheral tissue injury in infant rats, at an age

roughly equivalent to that of a human neonate. To characterize this circuitry, 12-day old rat pups were systemically injected with manganese chloride (75 mg/kg), a calcium analog able to pass through the blood brain barrier at this stage in development. Twenty-four hours later, half of the pups (n=19) were treated with formalin injected into the hindpaw and half were not (n=19). This injury model engages supraspinal pathways and has been used extensively in both developmental and adult studies as a model of inflammatory pain. To allow activation of multiple structures, pups survived for 7 hours prior to perfusion and brains were imaged for 8-10 hours *ex vivo*. T1 weighted 3D spin echo images were used to visualize the Mn uptake in the brain (Bruker Biospin). Images were normalized and smoothed to a previously established group template space (Advanced Normalization Tools) and intensity normalized to the visual cortex, a region unlikely to be affected by pain stimuli. Two methods of analysis were implemented to elucidate the pain circuits. Voxel and region-wise morphometric analysis showed statistically significant differences in several brain regions between the control and formalin-treated groups ($p < 0.001$, uncorrected for multiple comparisons). Using structural equation modeling (SEM) of anatomically defined circuits, and iterative addition and subtraction of anatomical components known to be involved in pain processing, two possible models that accounted for the maximum variance (comparative fit index, CRI > 0.90 and standardized root mean square residual, SRMR < 0.08) were identified. In these models the formalin-treated group exhibited a significant fit to the SEM, whereas the control group did not, suggesting that these pathways are involved in the pain perception circuitry, as activated by the formalin stimulus. These findings suggest that as young as 12 days of age, a painful injury activates both the sensory and affective components of pain.

Disclosures: **M.M. Sperry:** None. **B.M. Kandel:** None. **K.E. Bass:** None. **S.R. Das:** None. **P.S. Dhillon:** None. **J.C. Gee:** None. **G.A. Barr:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.18/A34

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NSERC/CIHR CHRP 414500-12

Title: Measuring the development of auditory-language function in the first year with functional MRI

Authors: ***C. J. WILD**[1], A. C. LINKE[1], L. ZUBIAURRE-ELORZA[2], C. HERZMANN[1], H. DUFFY[3], V. K. HAN[4], D. S. C. LEE[4], R. CUSACK[1,4];
[1]Western Univ., Brain and Mind Inst., London, ON, Canada; [2]Univ. of Deusto, Fac. of

Psychology and Educ., Bilbao, Spain; [3]Dept. of Psychology, Univ. of Warwick, Coventry, United Kingdom; [4]Children's Hlth. Res. Inst., Lawson Hlth. Res. Inst., London, ON, Canada

Abstract: The auditory perceptual abilities of newborn human infants develop rapidly as they are exposed to a rich acoustic environment that includes spoken language, but little is known about how the cortical auditory system - the substrate of these abilities - changes during this time of critical development. Functional neuroimaging studies have shown that it possible to measure brain responses to sounds in neonates and very young infants, but there have been no controlled studies to investigate what aspect of sounds auditory cortex (AC) is sensitive to, and there are no studies that examine how sensitivity to complex auditory features changes with age. In this study, we used functional magnetic resonance imaging (fMRI) to measure brain responses evoked by rich and naturalistic sounds at two time points during the first 12 months. 3-month (N=9) and 9-month old (N=11) infants, and adult control subjects (N=16), were scanned in two 8 minute blocks while listening to a series of lullabies. Vocal recordings were re-synthesized to create stimulus classes that varied in high-level auditory features (high vs. low phonemic content, and more vs. less human sounding), and importantly, were matched in basic acoustic properties (pitch, amplitude envelope, and frequency spectrum). Infants were a mixed group of term-born healthy infants (N=3 and N=4, 3- and 9-month olds) and NICU graduates. Univariate fMRI analysis showed reliable sound-evoked activity in AC in 3- and 9-month old subjects, but only adult AC was sensitive to the different classes of sounds. Inter-subject correlation (ISC) was used to measure the degree to which individuals within a group showed a similar response when listening to the same songs; unlike block-averaged comparisons between conditions, ISC can detect modulations of brain activity during stimulus presentation, and is therefore sensitive to differences in responses between individual songs, or even between different parts of the same song. We found that 3-month old listeners had significantly correlated responses in bilateral AC, cerebellum, and the supplementary motor area. Importantly, ISCs persisted when the neural responses were adjusted to control for the simple response to the presence of sound. This shows that at three months of age, AC is sensitive to the differences in features between sounds. Interestingly, no such inter-subject correlations were found at 9-months of age, despite the presence of auditory-evoked activity, which might reflect different feature sensitivity, attention, or developmental trajectories between individuals.

Disclosures: C.J. Wild: None. **A.C. Linke:** None. **L. Zubiaurre-Elorza:** None. **C. Herzmann:** None. **H. Duffy:** None. **V.K. Han:** None. **D.S.C. Lee:** None. **R. Cusack:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.19/A35

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: Battaglia Endowment to GAB

NIH Grant MH80603 to GAB

NIH Grant DC009910 to RMS

NIH Grant MH091451 to RMS

NIH Grant HD083217 to RMS

Title: Long and short term effects of the mother's presence during noxious stimulation in the infant rat

Authors: *G. A. BARR[1], R. PERRY[2], R. M. SULLIVAN[2];
[1]Anesthesiol. and CCM, Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Dept. of Child and Adolescent Psychiatry, New York Univ. Langone Med. Ctr., New York, NY

Abstract: Modern medical treatment allows very low birth weight premature infants to survive and hundreds of thousands of newborn infants are admitted to the neonatal intensive care unit in the United States yearly. Each infant is subjected to up to 10-20 daily medically essential painful procedures with analgesia used in fewer than half of these procedures. Increasingly, non-pharmacologic interventions are being utilized, where the caregiver provides physical comfort during procedures to reduce pain and stress, including kangaroo care and facilitated tucking. Although effective in reducing pain in these infants, limited clinical and basic science data suggest these well-intentioned interventions likely have long term consequences. As non-pharmacologic treatments continue to be implemented, it is important to understand the long term effects of caregiver comfort during painful procedures early in life. To test the long-term effects of maternal comfort on adult pain responses, we presented mild electric shocks (0.5mA) over a 45 minute period daily to the hindquarters of rat pups aged PN5-9 or PN10-14 with or without the mother present. We included a no-shock, no-mom control group. PN5-9 includes the pain sensitive period when infant inflammatory injury has long term effects on adult pain responses and the infant attachment period when pups learn preferences to odors associated with the shock. PN10-14 is outside the pain sensitive period and includes the attachment transitional period when pups learn aversions to odors paired with the shock if tested without their mother but preferences if tested with the mother. At both ages, escape behavior to the shock and ultrasonic vocalizations were reduced when the mother was present. Pups grew up and were regularly handled to reduce stress. At 50-60 days of age, adults were tested in the plantar thermal test for baseline latencies and on the next day, one hour following carrageenan inflammation of the hindpaw. There was a slight but statistically significant elevated withdrawal threshold (longer latency) in the adults who experienced pain with the mother present at PN10-14 compared to shock without the mother or the no-shock controls. There were no differences in the PN5-9 group. Following inflammation, withdrawal latencies were reduced in all groups at both ages and there was a significant decrease in the hyperalgesia induced by carrageenan in pups shocked with the mother at PN5-9 compared to the two other groups. No differences were found in the PN10-14 in the carrageenan test. The results show short and long-term reductions in inflammatory pain by the mother’s presence that is age specific during a painful experience.

Disclosures: **G.A. Barr:** None. **R. Perry:** None. **R.M. Sullivan:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.20/A36

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant NSO72454

Title: Analysis of proprioceptive sensory afferent inputs on populations of spinal interneurons in neonatal mice

Authors: ***D. R. LADLE**, B. GOSKY, T. RAPETTI, P. PAINTER, Y. DAI;
Neurosci, Cell Bio, Physiol, Wright State Univ., Dayton, OH

Abstract: A key step in the development of neural circuits is formation of synaptic connections with restricted subsets of neurons such that circuits with behaviorally appropriate output are produced. Synaptogenesis between primary afferents of the somatosensory system and neuronal targets in the spinal cord provides an attractive model system for such studies. Analysis of synaptic connections made by muscle spindle afferents on defined interneuron populations in the deep ventral horn may be particularly instructive. Among the interneurons in the ventral horn, two classes of inhibitory neurons, Ia inhibitory interneurons (IaINs) and Renshaw cells (RCs) have been reasonably well described in terms of morphology and function. Glycinergic IaINs mediate reciprocal inhibition whereby activation of muscle spindle afferents in a given muscle, an extensor muscle, for example, leads to inhibition of motor neurons that antagonize movement at the same joint (i.e. flexor muscles). RCs are primarily driven by motor axon collaterals and mediate recurrent inhibition. While monosynaptic connections between muscle spindle afferents and IaINs have long been demonstrated, monosynaptic connections between afferents and RCs have only recently been described in postnatal animals. To date, no studies have analyzed the selectivity of afferent connections with IaINs or RCs during development. Therefore, we mapped the connections of afferents of the quadriceps (Q; knee extensor) and obturator (Obt; supplying adductor muscles) nerves onto immunohistochemically defined IaINs and RCs, using retrograde tracing with fluorescent dextrans in an isolated spinal cord/muscle nerve preparation from neonatal mice (P0/P1). The percentage of identified interneurons in lumbar segments 3 and 4 contacted by either or both muscle afferent sources were quantified by analysis of high-magnification confocal image stacks. Similar percentages of IaINs received contacts from either Q (34 ± 4%; n = 3 animals) or Obt (36 ± 4%) afferents. A significant portion of IaINs, however, received converging contacts from both afferent sources (30 ± 5%). We observed half of RCs (56 ± 5%; n = 5 animals) do not receive contacts from Q or Obt afferents at this early stage. Among RCs contacted by Q or Obt afferents in these animals, 45 ± 2% received contacts from only Obt

afferents, while only 28 ± 3% were exclusively contacted by Q afferents. The remaining 26 ± 3% of RCs received convergent contacts from both muscle afferent sources. These results reveal a previously unappreciated degree of convergence of sensory input from disparate afferent populations on spinal interneurons in early postnatal animals.

Disclosures: D.R. Ladle: None. **B. Gosky:** None. **T. Rapetti:** None. **P. Painter:** None. **Y. Dai:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.21/A37

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: Medical Research Council funded PhD (ASD)

Title: Larval mutant ninja zebrafish: an axon initial segment *in vivo* imaging project

Authors: *A. S. DUMITRESCU, M. P. MEYER, M. S. GRUBB;
Kings Col. London, London, United Kingdom

Abstract: The axon initial segment (AIS) is a specialised neuronal compartment involved in the maintenance of axo-dendritic polarity and in the generation of action potentials. The AIS has also been shown to be a novel site for neuronal plasticity, as changes in ongoing activity levels *in vitro* and *in vivo* result in significant alterations in the structure's position or size thus regulating a cell's intrinsic excitability. However, all findings so far have been based on experiments carried out on mammalian fixed samples that offer only a snapshot view of this newly described plasticity mechanism. This approach is limited as it always compares and contrasts findings of AIS plasticity based on group averages and it doesn't allow us to follow individual cell dynamics. We are aiming to address this significant gap in knowledge and pursue the first AIS live imaging experiments making use of the zebrafish larvae for *in vivo* imaging, pioneering research at a time when there is almost nothing known regarding the AIS in this animal model. We have started by developing techniques to visualise the AIS in both fixed samples and live zebrafish. At the moment we have established protocols to confirm and describe AIS morphologies in zebrafish wholemount fixed tissue in retinal ganglion cells, tectal and cerebellar neurons. In parallel we have created and are now in the process of testing several zebrafish specific fluorescently tagged AIS live-imaging constructs. To further probe AIS function *in vivo* we have made use of the TALEN genome editing system to generate both a mutant Ankyrin-G knockout zebrafish line and a transgenic Ankyrin-G:EGFP insertion line. Using our newly developed tools we will fully exploit the power of this model organism to watch the AIS develop

and adapt, live and *in vivo* focusing on retinal ganglion cells during controlled manipulations of sensory experience.

Disclosures: A.S. Dumitrescu: None. **M.P. Meyer:** None. **M.S. Grubb:** None.

Poster

290. Development of Sensory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 290.22/A38

Topic: A.06. Development of Motor, Sensory, and Limbic Systems

Support: NSF Grant 10S-0924143

Title: Disabled-1 expression identifies a subset of Lmx1b superficial dorsal horn neurons involved in nociceptive circuits

Authors: *G. METTA YVONE, H. ZHAO, J. C. UDEOCHU, P. E. PHELPS;
Integrative Biol. and Physiol., UCLA, Los Angeles, CA

Abstract: Neuronal positioning defects are found in the dorsal horn of the spinal cord in the absence of Reelin, the two lipoprotein Reelin receptors (Vldlr and ApoER2), or the intracellular adaptor protein Disabled-1 (Dab1). These mispositioned neurons cause distinct sensory abnormalities: thermal (heat) hyperalgesia and a reduction in mechanical sensitivity. In this study we used mice with the *lacZ* reporter gene inserted into the *dab1* locus to identify correctly positioned Dab1 neurons in heterozygous ($dab1^{lacZ/+}$) mice and mispositioned neurons in mutant ($dab1^{lacZ/lacZ}$) mice. Initially we used sensory testing and cFos expression to verify that $dab1^{lacZ/+}$ mice replicate the sensory behavior of the $dab1^{+/+}$ mice and $dab1^{lacZ/lacZ}$ mice display the same nociceptive abnormalities as other Reelin-signaling pathway mutants. To identify the Dab1-labeled cells in laminae I-II (superficial dorsal horn), lateral lamina V, and the lateral spinal nucleus (LSN) in the lumbar enlargement, we combined β-galactosidase histochemistry with immunofluorescence. In the superficial dorsal horn, Dab1 rarely or never co-localizes with GAD67, Calbindin, Calretinin, or PKCgamma. However, about 70% of these Dab1-positive neurons co-express Lmx1b, a transcription factor that marks excitatory glutamatergic neurons in the dorsal horn. Many Dab1 cells in lateral lamina V and the LSN also co-localize with Lmx1b. The Dab1-Lmx1b neurons in the superficial dorsal horn appear subtly mispositioned, whereas those in lateral lamina V and LSN have extensive positioning errors. We then asked if neurons in the combined lateral cervical nucleus (LCN) and LSN, an area in high cervical segments that relays pain information from the spinal cord to the brainstem and thalamus, sustain positioning errors in Reelin-signaling pathway mutants. On average, there were 50% more neurons in $dab1^{+/+}$ than $dab1^{lacZ/lacZ}$ LCN/LSN. Taken together, these results suggest that migratory errors of

Dab1-expressing neurons in lumbar dorsal horn and the high cervical LCN/LSN likely contribute to the nociceptive defects found in Reelin-signaling pathway mutants.

Disclosures: **G. Metta Yvone:** None. **H. Zhao:** None. **J.C. Udeochu:** None. **P.E. Phelps:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.01/A39

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant R01 DC002167

Title: Potency and neurogenesis in the basal stem cells of the olfactory and respiratory epithelia

Authors: ***J. N. PETERSON**, J. SCHWOB;
Dept. of Cell, Mol. and Developmental Biol., Tufts Univ. Sackler Sch. of Grad. Biomed, Boston, MA

Abstract: The mammalian olfactory epithelium (OE) and neighboring respiratory epithelium (RE) both possess a remarkable capacity for repair and regeneration in response to injury. This regeneration is made possible in part through the action of two resident stem cell populations that closely resemble each other but are fated to give rise to different types of cell and exhibit different levels of activity. In the RE, these basal cells will give rise to ciliated columnar epithelium and mucous-producing goblet cells and are active and proliferative under normal physiological conditions. In the OE, the most basal stem cells are termed horizontal basal cells (HBCs) and will give rise to the olfactory sensory neurons and supporting sustentacular cells, but remain quiescent until activated by severe tissue injury. This project seeks to understand the differences in activity and plasticity between these two histologically and morphologically similar stem cell populations with an emphasis on what gives HBCs their unique neurogenic potential. To approach this question we utilize transgenic mice, a novel xeno-free *in vitro* system, an olfactotoxic lesion system and transplantation studies. We have identified the first unique histological marker that is expressed differentially between the two stem cell populations. Our *in vivo* and *in vitro* results indicate that this marker is functionally important in defining their relative plasticity and may be regulated by the physiological microenvironment. By exploring the differences between these two stem cell populations we hope to gain a better understanding of the nature of the regulation of tissue stem cell plasticity and potency.

Disclosures: **J.N. Peterson:** None. **J. Schwob:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.02/A40

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant F31 DC014398

NIH Grant R01 DC002167

Title: Location, location, location: neuronal diversification in the olfactory epithelium

Authors: *J. C. HEWITT[1], R. P. LANE[3], J. E. SCHWOB[2];
[1]Neurosci., [2]Developmental Mol. and Chem. Biol., Tufts Univ., Boston, MA; [3]Mol. Biol. and Biochem., Wesleyan Univ., Middletown, CT

Abstract: Mature olfactory sensory neurons (OSNs) express olfactory receptors (ORs) in specific zones along the dorsal/ventral axis of the olfactory epithelium (OE). In addition to the spatial patterning of OR expression, dorsomedial OSNs express the enzyme NQO1 exclusively while ventrolateral OSNs express the mammalian homologue of fasciclin II (mamFasII, or OCAM) exclusively; the dorsomedial and ventrolateral territories are sharply bounded and strictly complementary. During the reconstitution of the neuronal population following injury, the spatial patterning of OR expression is restored as is the boundary and complementarity between NQO1 and mamFasII/OCAM expression. The re-emergence of spatial organization across the OE may reflect positional “memory” in the spared, residual stem cells, or, alternatively, cues from surrounding tissues that do not suffer direct injury. As a test of these two alternatives, we tested whether neurons derived from transplanted stem/progenitor cells would retain the markers (OCAM/NQO1) and receptors (ORs) that characterize the region from which they were harvested, or adopt the phenotype of neurons that typify where they engrafted. Specifically, progenitors were harvested from both the dorsal and ventral OE of CAG-TdTomato transgenic mice and transplanted separately into regenerating epithelium of host male mice that had been exposed to the selective olfactotoxin methyl bromide the previous day. After recovering for three weeks, which provides adequate time for OSN regeneration, the OE was harvested. Immunohistochemical analysis indicated that progenitors transplanted from dorsal OE that engraft in ventral OE give rise to neurons that much more often express the ventral marker OCAM/mamFasII than the dorsal marker NQO1; 81% of all neurons that are born following dorsal into ventral transplant assume a ventral identity (n = 3 transplants; 1473 OSNs). Likewise, OR expression was determined by single cell RT-PCR on transplant-derived neurons isolated by FACS, and the spatial distribution of the harvested OR was assayed by *in situ* hybridization; 75% of the transplant-derived neurons express a ventrally located OR (n = 2 transplants; 4 neurons). These data suggest that most transplant-derived OSNs are instructed by cues that derive from the local environment rather than a memory of place. These data also raise the question as to why a

significant proportion of the OSNs do not demonstrate this plasticity. Follow-up studies will assess if stem cells at later stages of neuronal commitment are less spatially plastic during transplantation experiments.

Disclosures: J.C. Hewitt: None. **R.P. Lane:** None. **J.E. Schwob:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.03/A41

Topic: A.07. Transplantation and Regeneration

Support: College of Charleston Office of Undergraduate Research and Creative Activities

a grant to the College of Charleston from the Howard Hughes Medical Institute through the Pre-College & Undergraduate Science Education Program

Title: Estrogen signaling is necessary for the exercise-mediated increase in motoneuron participation in axon regeneration after peripheral nerve injury in mice

Authors: *J. C. WILHELM, P. A. COPLEY, M. C. ACOSTA, J. R. HARRELL;
Col. of Charleston, Charleston, SC

Abstract: Thousands of people each year suffer from peripheral nerve injury. Treatment options are limited, and recovery is often incomplete. Treadmill exercise can enhance nerve regeneration; however, this appears to occur in a sex-dependent manner. Females respond best to short duration, high speed interval training; whereas, males respond best to slower, continuous training. Previous studies have shown a role for testosterone in this exercise-mediated enhancement, but the role of estrogen is unknown. To evaluate the role of estrogen signaling in treadmill exercise, we treated male and female wild type mice with an estrogen receptor antagonist, ICI 182,780 during exercise. The right common fibular branch of the sciatic nerve was cut and repaired with fibrin glue. Estrogen-filled or blank capsules were implanted subcutaneously at the time of nerve transection. Starting three days post-transection, exercised mice received treadmill training using the paradigm appropriate to their sex 5 days a week for 2 weeks. Fourteen days after the initial sciatic nerve transection, motoneurons whose axons had regenerated at least 1500 μm distal to the original cut sites were labeled with a retrograde tracer. Regeneration was quantified by counting the number of fluorescent labeled motoneurons in the lumbar region of the spinal cord. Both treadmill training and estrogen administration increased the number of motoneurons participating in axon regeneration, but this effect was blocked by estrogen receptor antagonist treatment. Estrogen signaling is important for the enhancing effects of treadmill exercise on motoneuron participation after peripheral nerve cut.

Disclosures: **J.C. Wilhelm:** None. **P.A. Copley:** None. **M.C. Acosta:** None. **J.R. Harrell:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.04/A42

Topic: A.07. Transplantation and Regeneration

Title: Neuropeptide Y induces hematopoietic stem cell mobilization and improves bone loss

Authors: ***H. JIN**[1], N. KIM[1], M. PARK[2], J.-S. BAE[2];
[2]Sch. of Med., [1]Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: Hematopoietic stem cell (HSC) mobilization is an essential homeostatic process regulated by the interaction of cellular and molecular components in bone marrow niches. It has been shown by others that neurotransmitters released from the sympathetic nervous system regulate HSC egress from bone marrow to peripheral blood. In this study we investigate the functional role of neuropeptide Y (NPY) on this process. NPY deficient mice had significantly impaired HSC mobilization due to increased expression of HSC maintenance factors by reduction of matrix metalloproteinase-9 (MMP-9) activity in bone marrow. Pharmacological or endogenous elevation of NPY led to decrease of HSC maintenance factors expression by activating MMP-9 in osteoblasts, resulting in HSC mobilization. Mice in which the Y1 receptor was deleted in osteoblasts did not exhibit HSC mobilization by NPY. Furthermore, NPY treatment in ovariectomized mice caused reduction of bone loss due to HSC mobilization. These results suggest a new role of NPY on HSC mobilization, as well as the potential therapeutic application of this neuropeptide for stem cell-based therapy.

Disclosures: **H. Jin:** None. **N. Kim:** None. **M. Park:** None. **J. Bae:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.05/A43

Topic: A.07. Transplantation and Regeneration

Support: AIHS Bridge Funding (Operating)

Seed Grant (Operating) 1024536

Title: AlphaB-crystallin does not alter the immune response after sciatic nerve crush injury

Authors: *E.-M. F. LIM[1], V. HOGHOOGHI[1], S. S. OUSMAN[2];
[1]Neurosci., [2]Clin. Neurosciences, Univ. of Calgary, Calgary, AB, Canada

Abstract: During Wallerian degeneration (WD) in the injured peripheral nervous system (PNS), immune cells play a critical role in creating a supportive environment for regrowing axons. Dedifferentiated Schwann cells secrete cytokines and chemokines such as TNFα, IL-1α, IL-1β, MCP-1 and MIP-1α that promote the recruitment of monocytes to the injured site. These monocytes differentiate into macrophages and produce TNFα, IL-1α and IL-1β, which contribute to further recruitment of hematogenous macrophages and the breakdown of myelin. Importantly, these hematogenously derived macrophages phagocytose axonal and myelin debris that are inhibitory to axon growth. The beneficial aspects of cytokines and immune cells are evident, but a prolonged inflammatory response after injury has been implicated in the pathogenesis of negative symptoms after peripheral nerve injury, specifically neuropathic pain, and therefore must be carefully controlled to prevent damage and allow for subsequent regeneration. AlphaB-crystallin (αBC) is a small heat shock protein that has been shown to have chaperone properties, promote survival of various peripheral cells undergoing stress and, decrease inflammation. Further, αBC is expressed in the PNS and we have previously shown its beneficial role in peripheral nerve regeneration where mice null for the crystallin have thinner myelin sheaths, decreased conduction velocity, and impaired motor and sensory functions. Due to the extensively reported immunomodulatory role of αBC, we investigated whether the heat shock protein influenced PNS regeneration by modulating the immune response during WD. The *in vivo* cytokine profiles of TNFα, IL-1β, and IL-6 in the distal nerve segment of 1-28d post-crushed WT and αBC-/- mice were found to be similar between the two groups. Further, the number of CD45 and Iba1 positive profiles was also not altered between WT and αBC-/- mice at 1-7 days post-injury. To determine whether αBC can affect the level of cytokines secreted by macrophages, we stimulated these cells *in vitro* with LPS in the presence or absence of recombinant human αBC and evaluated the levels of IL-12p40, IL-1β, TNFα, IL-6 and IL-10. We observed a decrease in levels of IL-12, IL-1β, and TNFα and an augmentation of IL-10 secretion following treatment with αBC. In this study, we show for the first time that treating macrophages with αBC can decrease the secretion of pro-inflammatory cytokines and increase the secretion of anti-inflammatory cytokines *in vitro*. However, in the peripheral nerve crush injury paradigm, αBC does not modulate the immune system to affect regeneration which suggests specificity in the role of the crystallin after PNS injury.

Disclosures: E.F. Lim: None. **V. Hoghooghi:** None. **S.S. Ousman:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.06/A44

Topic: A.07. Transplantation and Regeneration

Support: R01 DC002167

F30 DC013962

Title: Notch signaling helps maintain reserve neural stem cell quiescence in the setting of neuronal injury

Authors: *D. B. HERRICK[1], J. E. SCHWOB[2];
[2]Developmental Mol. and Chem. Biol., [1]Tufts Univ., Boston, MA

Abstract: The olfactory epithelium (OE) retains the capacity - remarkable for the nervous system - to repair itself structurally and functionally after wholesale or piecemeal loss of olfactory neurons. As such, the OE can regenerate olfactory sensory neurons (OSNs) and all nonsensory cellular constituents, including sustentacular (Sus) cells, throughout adult life by recruiting neurocompetent stem and progenitor cells, particularly in response to epithelial injury. Thus, the OE is an attractive and powerful model for studying adult neurogenesis. Two types of neurocompetent stem cells function within the OE: dormant horizontal basal cells (HBCs), a reserve population, and constitutively active globose basal cells (GBCs). Our lab and others have demonstrated that down-regulation of ΔNp63α, a member of the p53 family of transcription factors, is necessary and sufficient to elicit HBC activation. However, the molecular signaling events upstream of HBC activation remain poorly understood. RNA-seq analysis of purified HBCs following injury demonstrated that the Notch signaling pathway, an evolutionarily conserved cell-cell signaling pathway, is a crucial regulator of HBC activation after injury. Notch receptors and canonical Notch-dependent signaling targets were both down-regulated following injury, which coincides with down-regulation of ΔNp63α mRNA. Genetic deletion of Notch1 in HBCs *in vivo* produces a decrease in ΔNp63α mRNA and increased activation to multipotency as demonstrated by HBC lineage tracing, whereas conditional overexpression of Notch1 intracellular domain (which drives expression of Notch targets) leads to an increase in ΔNp63α expression. Selective death of neurons following olfactory bulb ablation (OBX) causes increased Notch receptor levels by qRT-PCR and immunohistochemistry but was not sufficient to activate HBCs to multipotency. However, OBX in the setting of HBC-specific knockout of Notch1 does activate at least some HBCs to multipotency as shown by tracing the descendants of the recombined HBCs. The resultant clones are similar in composition to those generated in response to MeBr injury, wherein wholesale loss of neurons and Sus cells by MeBr injury results in both decreased Notch signaling and arousal out of dormancy. Thus, Notch1 is required to maintain HBC quiescence in the setting of neuronal injury, suggesting that the undamaged Sus cells play a significant role in maintaining HBC quiescence via Notch signaling. The current results implicate the Notch signaling pathway as a potential target for manipulation in dormant neuronal stem cell populations following injury. Supported by NIH grants R01 DC002167 and F30 DC013962

Disclosures: **D.B. Herrick:** None. **J.E. Schwob:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.07/A45

Topic: A.07. Transplantation and Regeneration

Support: National Key Basic Research Program of China [2014CB542202]

863 Program [2012AA020502]

National Natural Science Foundation of China [81130080, 81171180, 81371389 and31200799]

the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD)

Title: MiR-9 inhibits Schwann cell migration by targeting Cthrc1 followingsciatic nerve injury

Authors: S. ZHOU, D. SHEN, Q. ZHANG, H. SHI, X. GU, *F. DING;
Nantong University, China, Jiangsu, China

Abstract: After peripheral nerve injury (PNS), the proximal nerve stump will spontaneously regenerate due to the biological behaviors of Schwann cells, the principal glial cells in the PNS. microRNAs (miRNAs), a class of approximately 22 nucleotide non-coding RNA molecules, negatively regulate the expression of a wide variety of genes mainly through a direct interaction with the 3'-untranslated regions (3'-UTRs) of their corresponding mRNA targets. Collagen triple helix repeat containing protein 1 (Cthrc1) has been shown to be overexpressed in injured arteries of rats, and Cthrc1 increases cellular motility to repair the injury by promoting cell migration. In this study, miR-9 was identified as an important functional regulator of Schwann cell migration that was a crucial regenerative response of Schwann cells to nerve injury. *In vitro*, upregulated expression of miR-9 inhibited Schwann cell migration, whereas silencing of miR-9 promoted Schwann cell migration. Intriguingly, miR-9 exerted this regulative function by directly targeting Cthrc1, which in turn inactivated downstream Rac1 GTPase. Rac1 inhibitor reduced the promotive effects of anti-miR-9 on Schwann cell migration. *In vivo*, high expression of miR-9 reduced Schwann cell migration within a regenerative nerve microenvironment. Collectively, our results confirmed the role of miR-9 in regulating Schwann cell migration after nerve injury, thus offering a new approach to peripheral nerve repair.

Disclosures: **S. Zhou:** None. **D. Shen:** None. **Q. Zhang:** None. **H. Shi:** None. **X. Gu:** None. **F. Ding:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.08/A46

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant K08DC008109

Title: Olfactory bulb targeting after recovery from olfactory epithelial injury

Authors: *E. H. HOLBROOK[1,2], A. R. DEZUBE[2], J. E. SCHWOB[2];
[1]Mass Eye & Ear Infirmary, Boston, MA; [2]Developmental, Mol. and Chem. Biol., Tufts Univ. Sch. of Med., Boston, MA

Abstract: In mice, each set of olfactory receptor (OR)-defined olfactory sensory neurons (OSNs) converge their axons and synapse within an average of 2 to 3 glomeruli per olfactory bulb (OB). The maintenance of this map is crucial for normal olfactory function. Although the robust regenerative capacity of the olfactory epithelium (OE)(including spatially-appropriate restoration of OR-defined OSNs) is well-established following injury, evidence suggests that glomerular targeting may be inaccurate and aberrant during reinnervation, thereby causing long-lasting olfactory dysfunction. Thus, disorganization in the projection from the OE to the OB is suggested in humans by the common complaints of phantosmia and parosmia during recovery from head trauma and other epithelial insults. We have previously reported significant errors in glomerular targeting after recovery from MeBr-induced epithelial injury using the P2-ITL mouse line, which genetically labels P2 receptor-expressing OSNs (Holbrook et al., 2014). These abnormalities include both incomplete and multiple innervation of glomeruli. In order to assess the pervasiveness of errors in targeting after recovery from OE lesion, we compared axonal targeting after regeneration vs. control in the additional receptor-tagged mouse lines M72-ITG and I7-ITG as well as P2-ITL. We occluded one naris in mice (n = 3) from each of the three transgenic lines during the 8-hour period of exposure to MeBr gas (175 ppm) to create unilateral epithelial lesions. The nasal plug was removed at completion of exposure, and the mice survived in their usual housing for eight weeks to allow for maximal recovery of the OE and reinnervation of the OB. Coronal cryosections through the olfactory epithelium and OB were immuno-labeled for beta-galactosidase (P2) or GFP (M72 and I7). Reconstructed mosaic images of the OB sections were analyzed for glomerular targeting including number and completeness of glomerular innervation by comparison with olfactory marker protein (OMP)-staining. Our findings indicate a differential capacity for accurate reinnervation depending on the type of OSN that is assessed. The types of targeting errors made by P2 OSNs were not observed to the same degree as M72- or I7 OSNs. The explanation for the results may be due to differences in sensory activation of the different types of OSNs within the laboratory environment. The results are important in extending our understanding of the regenerative capacity of the olfactory system

beyond the OE recovery. In addition, our findings indicate the need to analyze multiple OSN types to achieve a fuller sense of the outcome of the regenerative process.

Disclosures: **E.H. Holbrook:** None. **A.R. Dezube:** None. **J.E. Schwob:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.09/A47

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant R01 DC140217

Title: A transgenic mouse model for accelerated olfactory aging

Authors: *W. JANG[1], E. HOLBROOK[1,2], K. CHILD[1], J. SCHWOB[1];
[1]Tufts Univ. Sch. of Med., Boston, MA; [2]Massachusetts Eye and Ear Infirmary, Harvard Med. Sch., Boston, MA

Abstract: As a consequence of aging, the human olfactory epithelium (OE), which forms a continuous neuroepithelial sheet densely populated by olfactory sensory neurons (OSNs) at birth, becomes interrupted by patches lacking neurons. The aneuronal holes are due either to 1) metaplastic replacement by respiratory epithelium (RE) or 2) neurogenic exhaustion wherein the epithelium consists solely of sustentacular cells and horizontal basal cells (HBCs) and is completely lacking in OSNs and globose basal cells (GBCs). We have identified a transgenic mouse model of enhanced OE aging due to accelerated neuronal turnover as a consequence of which the OE undergoes similar pathological changes as in human OE. Accelerated turnover occurs in the OE of a bigenic mouse line combining an OMP-tTA knock-in mutation ("Tet-Off" tetracycline transactivator) and a TetO-DTA transgene (tet response element-diptheria toxin subunit A). In the absence of Doxycycline, expression of DTA in mature, OMP (+) OSNs abbreviates their lifespan and accelerates their death. Our immunohistochemical analysis demonstrates that the epithelium initially responds by increasing both GBC proliferation and neuron production. However, beginning around 2-4 months of age, neurogenesis declines, GBCs disappear - including the subset of upstream stem cells characterized by the expression of Sox2 and Pax6, and the epithelium becomes progressively aneuronal. On the other hand, HBCs, a population of neurocompetent stem cells that are held in reserve, express high levels of the transcription factor p63, remain dormant, and do not contribute to the repopulation of the epithelium. In addition, areas of the epithelium that were olfactory undergo respiratory metaplasia, a finding that had only been triggered by severe epithelial injury in the past. These areas are often associated with an increased infiltration by inflammatory cells. Analysis of the olfactory bulb (OB), the central target of the OSNs, demonstrates a reduction in glomerular

innervation and a down-regulation of tyrosine hydroxylase in the dopaminergic periglomerular neurons, consistent with their relative denervation. While some of these changes have been noted following other experimental manipulations, epithelial pathology emerges in those models only at a very advanced age. The accelerated aging of the OE in the current model will allow a more in-depth analysis of the age-associated shifts in the GBC population that precede neurogenic collapse and of the potential for ameliorating, preventing, or reversing the pathologies - both in the OE and the OB - that accompany the march of time.

Disclosures: **W. Jang:** None. **E. Holbrook:** None. **K. Child:** None. **J. Schwob:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.10/A48

Topic: A.07. Transplantation and Regeneration

Support: Med-El, Inc

NIH grant RO1-DC008429

Title: Stimulation of a denervated laryngeal muscle with low frequency promotes selective reinnervation and restores function

Authors: ***Y. LI**[1], S. HUANG[2], D. ZEALEAR[2];
[1]Hearing and Speech Sci., Vanderbilt Univ. Med. Center/Oto, Nashville, TN; [2]Otolaryngology, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Previously, electrical stimulation of a denervated canine laryngeal muscle was shown to promote reinnervation by original over foreign motoneurons. An implantable nerve stimulation-EMG system was used to index the appropriateness of reinnervation of the vocal fold abductor (posterior cricoarytenoid, PCA) muscle by inspiratory versus foreign reflex glottis closure (RGC) motoneurons following recurrent laryngeal nerve section and repair. In the present study in ten canines, a clinical model was used, where both nerves were sectioned and ventilation compromised due to loss of abduction. The EMG system and a pulse generator were implanted, the latter for electrical conditioning of PCA muscles. After nerve section, animals were randomly assigned to four groups to assess the effect of different muscle stimulus paradigms on reinnervation quality and degree of functional recovery: 1)40 pps train, 2)20 pps train 3)10 pps train and 4)control-no stimulation. One msec pulses were applied with 4 sec on/4 sec off duty cycle during the post neurorrhaphy regeneration period. In bimonthly sessions, spontaneous vocal fold movement was measured endoscopically during induced hypercapnea in the anesthetized animal. Rectified integrated EMG potentials were recorded from abductor muscles and adductor (thyroarytenoid, TA) muscles. Recordings were obtained during

hypercapnic respiration to index reinnervation by inspiratory motoneurons, and during superior laryngeal nerve stimulation to index reinnervation by RGC motoneurons. Exercise tolerance was measured on a treadmill in the awake animal using pulse oximetry. Results demonstrated that nonstimulated controls, 40 pps stimulated and 20 pps stimulated animals had faulty reinnervation (EMG), severe paradoxical closure of the glottis during hypercapnea, and poor tolerance to exercise. In contrast, stimulated 10 pps animals showed a near normal pattern of low PCA and high TA reinnervation by RGC motoneurons, minimal paradoxical glottis closure, and near normal exercise tolerance (12 minutes up to 8 mph). It would appear that low-frequency stimulation of a denervated PCA muscle simulating endogenous activity inhibits reinnervation by foreign RGC motoneurons, leaving receptor sites available for native inspiratory motoneurons. As a consequence, reinnervation of adductor muscles by inspiratory neurons is depressed; paradoxical glottis closure is reduced and exercise tolerance restored.

Disclosures: Y. Li: None. **S. Huang:** None. **D. Zealear:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.11/A49

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant R01DC002167 (JES)

NIH Grant F31DC014637 (BL)

Title: Injury can induce neuronally committed Neurog1+ progenitors to become multi-potent

Authors: *B. LIN[1], J. HEWITT[2], J. PETERSON[1], J. E. SCHWOB[1];
[1]DMCB, [2]Dept. of Neurosci., Tufts University, Sackler Sch., Boston, MA

Abstract: The mammalian olfactory epithelium (OE) is a powerful model system for studying neural regeneration due to constant turnover and genesis of mature neurons well through adulthood. Two broadly defined basal stem cell populations, globose basal cells (GBCs) and horizontal basal cells (HBCs), participate in regeneration after injury. GBCs are a heterogeneous population that encompasses Sox2/Pax6(+) multipotent cells, neuron-fated Ascl1(+) that are transit-amplifying elements, and Neurogenin1/NeuroD1(+) immediate neuronal progenitors. This hierarchical model of GBC fate is based on the epistatic consequences of gene knockout and temporal correlation during recovery after epithelial injury. As expected, transplantation of Neurog1+ GBCs from an uninjured donor yielded only neurons. Unexpectedly, Neurog1+ GBCs isolated from a setting of constant neuronal death and regeneration (olfactory bulbectomy) revealed a previously unanticipated plasticity as they generated clones containing not only neurons, but also non-neuronal lineage sustentacular cells as well as duct/gland units.

Consequently, we performed a short term lineage trace using the Neurog1-eGFP animal in the context of two independent injury models, showing co-labeling of eGFP with Sox2 and Pax6, which suggests a de-differentiative pathway. Importantly, genetic lineage tracing using both a Neurog1-CreER as well as a more upstream Ascl1-CreER driver has demonstrated a similar plasticity after injury *in situ*, suggesting that this is not due to stresses experienced during the transplantation assay. These results provide an indication that the apparent hierarchy of neuronal differentiation in the OE merely implies differentiative fate-not commitment.

Disclosures: B. Lin: None. **J. Hewitt:** None. **J. Peterson:** None. **J.E. Schwob:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.12/A50

Topic: A.07. Transplantation and Regeneration

Title: Neuropeptide Y regulates the hematopoietic stem cell microenvironment and prevents nerve injury in the bone marrow

Authors: *J.-S. BAE[1], M. PARK[1], N. KIM[1], H. JIN[2];
[1]Sch. of Med., [2]Col. of Vet. Med., Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: Many reports have revealed the importance of the sympathetic nervous system (SNS) in the control of the bone marrow environment. However, the specific role of neuropeptide Y (NPY) in this process has not been systematically studied. Here we show that NPY deficient mice have significantly reduced hematopoietic stem cell (HSC) numbers and impaired regeneration in bone marrow due to apoptotic destruction of SNS fibers and/or endothelial cells. Furthermore, pharmacological elevation of NPY prevented bone marrow impairments in a mouse model of chemotherapy-induced SNS injury, while NPY injection into conditional knockout mice lacking the Y1 receptor in macrophages did not relieve bone marrow dysfunction. These results indicate that NPY promotes neuroprotection and restores bone marrow dysfunction from chemotherapy-induced SNS injury through the Y1 receptor in macrophages. They also reveal a new role of NPY as a regulator of the bone marrow microenvironment, and highlight the potential therapeutic value of this neuropeptide.

Disclosures: J. Bae: None. **M. Park:** None. **N. Kim:** None. **H. Jin:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.13/A51

Topic: A.07. Transplantation and Regeneration

Support: Basic Science Research Program 2012-011783

Priority Research Centers Program 2009-0093829

Korea Health Technology R&D Project HI14C0522

Title: Administration of ursolic acid promotes axon regeneration by activating akt/s6 pathway after peripheral nerve injury

Authors: *J.-W. KIM[1,2,3], M. KIM[1,3,2], J. HYUN[1,2,3,4];
[1]Dankook Univ., Cheonan, Korea, Republic of; [2]Dept. of Nanobiomedical Sci. and BK21 PLUS NBM Global Res. Ctr. for Regenerative Medicine, Dankook Univ., Cheonan, Korea, Republic of; [3]Inst. of Tissue Regeneration EngineeringInstitute of Tissue Regeneration Engineering, Dankook Univ., Cheonan, Korea, Republic of; [4]Dept. of Rehabil. Medicine, Col. of Medicine, Dankook Univ., Cheonan, Korea, Republic of

Abstract: Ursolic acid (UA), a natural pentacyclic triterpene acid, has been reported to possess many biological functions, including anti-oxidant, anti-cancer, anti-inflammatory effects, and neuro-protective effect. However, no studies have been designed to investigate whether UA has an effect on axonal regeneration. Herein, we show here that administration of UA increases neurite outgrowth of rat dorsal root ganglion (DRG) neurons *in vitro* and promotes the regeneration of sciatic nerve *in vivo*. UA (2.5μM) promoted neurite outgrowth from primary cultured adult rat DRG neurons, whereas significantly decreased the number of axon branches. The effect of UA on neurite outgrowth of DRG neurons was similar with PTEN inhibitor bpV(HOpic). Combination of UA and bpV(HOpic) (UA+bpV(HOpic)) showed a synergistic effect on neurite outgrowth than UA only and bpV(HOpic) only, but no difference was found in the number of axon branches. Western blot results showed that phosphorylation level of AKT and S6 ribosomal protein was significantly upregulated in both UA and UA+bpV(HOpic) combination. Sciatic nerve crush injury was performed 5mm distal from the hip joint. The animals were randomly divided into four groups (n=5) : Group I, vehicle (corn oil, 0.25ml/day) ; Group II, UA (10 mg/kg/day); Group III, bpV(HOpic) (0.05 mg/kg/day); and Group IV, UA+bpV(HOpic) combination. Vehicle and drugs were intraperitoneally administered at 0 hr, 24 hr, and 48 hr. Assessment of regeneration using the pinch test 72 h after crush injury revealed that Group II, Group III, and Group IV were more effective compared to Group I in the rate of regeneration after sciatic nerve crush injury, but no significant difference was found among the 3 groups. Our findings indicate that UA can increase neurite outgrowth by upregulating phosphorylation level of AKT and S6 ribosomal protein and promote axonal regeneration following peripheral nerve injury.

Disclosures: **J. Kim:** None. **M. Kim:** None. **J. Hyun:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.14/A52

Topic: A.07. Transplantation and Regeneration

Support: SHRF 2668

SHRF 2921

CIHR MOP74747

UofS COM

Title: Acute intermittent hypoxia promotes regeneration-associated gene expression in axotomized peripheral nerve akin to electrical stimulation

Authors: *J. R. NADEAU[1,2], B. M. ARNOLD[2,1], G. D. MUIR[2], V. M. K. VERGE[1];
[1]Anat. and Cell Biology/CMSNRC, [2]Vet. Biomed. Sciences/WCVM, Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Despite an intrinsic ability for peripheral nerves to regenerate, it is still fraught with challenges often maladaptive in nature. However, the intrinsic repair mechanism can be greatly improved by brief electrical stimulation (ES) at the time of surgical repair resulting in better functional outcomes in both rodents and humans (Al-Majed et al. [2000] J. Neurosci. 20(7):2602-2608); Gordon et al. [2010] Exp. Neurology 223(1):192-202). However, ES is invasive and becomes difficult if the nerve is not readily accessible. An emerging non-invasive therapy, acute intermittent hypoxia (AIH), promotes respiratory and non-respiratory motor function in spinal cord injured rats and humans (reviewed in Dale et al., [2014] Physiology 29(1):39-48). AIH consists of breathing alternating periods of air with half oxygen (11%) and regular air for ten cycles and results in increased phrenic nerve output activity in the rat (reviewed in Mitchell GS, Johnson SM. [2003] J Appl Physiol. 2003;94:358–374). Because the AIH effect is presumably systemic and likely also increases neuronal activity in the peripheral nervous system, we hypothesized that it will impact repair of peripheral nerves and regenerative neuronal reprogramming in a manner akin to that we have observed with ES. To examine the impact of AIH on regeneration-associated gene expression, the tibial nerve in adult male Lewis rats was transected and repaired. AIH (5 min alternating for 10 cycles) was delivered on day 2 and day 3 post-tibial nerve repair, followed by animal perfusion and tissue dissection. Normoxia (continuous regular air) or sciatic nerve ES (1hr; 20Hz) delivered proximally at the time of nerve repair served as controls. Our preliminary results reveal a significant effect of AIH on regeneration associated-gene expression in a manner ressembling that in response to ES. Elevated levels of betaIII-tubulin, brain-derived neurotrophic factor, growth-associated protein-

43, SCG10 in the peripheral nerve neurons at the level of the cell bodies and the growing axon front were observed. Further, behavioral analysis of neuropathic pain states revealed no significant impact of either AIH or ES compared to normoxia for up to 10 weeks post-surgical repair. Impacts on axon regeneration are currently being assessed.

Disclosures: **J.R. Nadeau:** None. **B.M. Arnold:** None. **G.D. Muir:** None. **V.M.K. Verge:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.15/A53

Topic: A.07. Transplantation and Regeneration

Support: KAKEHIN B 25293137

KAKEHIN C 15K08684

KAKEHIN C 25460729

Title: Involvement of c-jun N-terminal kinase in neurite extension of cultured DRG neurons

Authors: **T. H. NGUYEN**, S. MATSUMURA, T. KATANO, *S. ITO;
Kansai Med. Univ., Hirakata Osaka, Japan

Abstract: Peripheral neurons are able to regenerate after axonal injuries. However, the quality of the peripheral nerve regeneration depends on the distance between the injured sites and the target organs. The longer the distance from the injured site to the target organs, the poorer the nerve regeneration. So, the speed of axonal outgrowth is important for the peripheral neurons to reach their target organs timely before these organs become impermissive for nerve regeneration. Neurotrophic factors including NGF, BDNF and GDNF are proved to accelerate peripheral nerve regeneration. We have established the sciatic nerve transection-regeneration model and showed that c-jun N-terminal kinase (JNK), a member of mitogen-activated protein kinase, delayed peripheral nerve regeneration. In an initial approach to clarify which neurotrophic factor is involved in peripheral nerve regeneration *in vivo*, we examined the effect of JNK of neurite extension using mice primary cultured DRG cells. NGF and GDNF at 10 ng/mL increased the percentage of neurons with neurites 3 times higher as compared to the control. Similarly, BDNF at 10 ng/mL increased it 1.5 times. The JNK 600125 inhibitor attenuated the increase in percentage of neurons with neurites by NGF, GDND, and BDNF up to 10 μM in a concentration-dependent manner. The maximum effect of inhibition was 4.4, 2.2 and 2.6 times for NGF, GDNF and BDNF, respectively. These results support the *in vivo* observation that neurotrophic factors may accelerate peripheral nerve regeneration through the JNK pathway.

Disclosures: **T.H. Nguyen:** None. **S. Matsumura:** None. **T. Katano:** None. **S. Ito:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.16/A54

Topic: A.07. Transplantation and Regeneration

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong, Special Administrative Region Government (01122016)

Title: Chronic effect of Pacific Ciguatoxin-1 on axonal regeneration and functional synapse formation after peripheral nerve injury

Authors: ***G. KUMAR**[1], P. ASTHANA[1], N. P. B. AU[1], C. TIN[2], Y. L. MAK[3,4], L. L. CHAN[1,3,4], P. K. S. LAM[3,4,5], C. H. E. MA[1,3];
[1]Dept. of Biomed. Sci., City University of Hong Kong, Kowloon, Hong Kong; [2]Dept. of Mechanical and Biomed. Engin., City University of Hong Kong, Kowloon, Hong Kong; [3]State Key Lab. in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong; [4]Shenzhen Key Lab. for the Sustainable Use of Marine Biodiversity, Res. Ctr. for the Oceans and Human Health, City Univ. of Hong Kong Shenzhen Res. Inst., Shenzhen, China; [5]Dept. of Biol. and Chem., City University of Hong Kong, Tat Chee Avenue, Hong Kong

Abstract: Ciguatera fish poisoning (CFP) is a common foodborne illness causing chronic and persistent neurological effects on peripheral nervous system. It has become a global health issue and its incidence is increasing. The initial symptoms of CFP are gastrointestinal and cardiovascular, which disappear by two weeks' time. However, neurological symptoms, such as muscle fatigue could last for months or years. Proximal peripheral nerve injuries could be potentially devastating and leading to motor function deficit. Restoration of motor function after injury requires not only successful regeneration of damaged axons, but also reinnervation to their target muscles, forming functional neuromuscular junctions (NMJs). Our behavioral studies showed that exposure to pacific ciguatoxin-1 (P-CTX-1) delayed sensory and motor function after sciatic nerve injury significantly. We therefore examined the chronic effect of P-CTX-1 on axonal regrowth and functional NMJ formation in mice after injury following pre-exposure to P-CTX-1. Male adult C57BL/6 mice were administered sub-lethal dose of P-CTX-1 (0.26ng/g, i.p.) twice (day 0 & 3) and sciatic nerve crush was performed on day 14. Animals were provided with food and water ad libitum and maintained under a 12:12 h light/dark cycle for 8 weeks. Sciatic nerve and lateral plantar muscles were harvested after perfusion two months after the injury. Transverse cryosection of sciatic nerve (Ipsilateral and contralateral) were immunostained with anti-NF-200 and total number of axons was quantified. Lateral planter muscle cryosection were immunostained with anti-α-bungarotoxin and anti-NF-200. Sections were quantified into three

categories i.e. fully innervated, partially innervated and denervated. The result of axon quantification showed significant decrease in NF-200 positive axon in the sciatic nerve 5- to 20-mm distal to injury site as compared with vehicle control in ipsilateral side ($P<0.05$). The number of axons in contralateral side reduced suggesting the systemic effect of P-CTX-1 on uninjured axons. NMJ numbers decreased significantly in the ipsilateral ($P<0.05$) and contralateral ($P<0.05$) side to injury in lateral planter muscle. We also performed electromyography (EMG) recording during the course of recovery to assess functional synapse formation following injury. EMG signal was reduced significantly in P-CTX-1 treated mice which in line with our histology and behaviour data.

Disclosures: **G. Kumar:** None. **P. Asthana:** None. **N.P.B. Au:** None. **C. Tin:** None. **Y.L. Mak:** None. **L.L. Chan:** None. **P.K.S. Lam:** None. **C.H.E. Ma:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.17/A55

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant DK097223

NIH Grant NS067431

NIH Grant P30 Y11373

Title: A peripheral compensatory mechanism of nerve debris clearance after injury in the absence of CCR2+ macrophages

Authors: *J. LINDBORG, R. ZIGMOND;
Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Wallerian degeneration is the process by which transection or crushing of the axons of peripheral neurons leads to degeneration and clearance of the distal axonal segment. Studies on Wallerian degeneration widely report that monocyte entry into the degenerating distal nerve is necessary for phagocytosis and is required to promote regeneration by the proximal nerve segment. CCR2+ monocytes enter tissues and differentiate into macrophages, which are purported to be the key players in this clearance process. Using a CCR2-/- mouse model, in which the infiltration of CCR2+ monocytes is inhibited, we found that these macrophages may not play as pivotal a role in axonal degeneration as previously believed. Specifically, the disappearances in the distal nerve of myelin (including a specific myelin protein P0) and the axonal light neurofilament protein were similar in CCR2-/- and in wild type (WT) mice 7 days after a sciatic nerve transection, in spite of substantially decreased macrophage accumulation in

the CCR2-/- nerve. Luxol fast blue and toluidine blue-stained nerves were used to assess myelin degeneration and removal after nerve transection. A phagocytosis assay and a time course after injury show that significantly more myelin is removed at 3 days post-injury in the distal nerve segment of CCR2-/- mice, and that an increased amount of myelin is contained within glial fibrillary acidic protein (GFAP)-labeled Schwann cells compared to WT mice. Flow cytometry, western blotting, qPCR, and immunohistochemistry were used to identify the fluctuating patterns of phagocytosis by leukocytes and Schwann cells during degeneration of the sciatic nerve in WT and CCR2-/- animals. We hypothesize that in the absence of infiltrating CCR2+ monocytes in the CCR2-/- mouse, a different phagocyte (or phagocytes) plays a compensatory role in the clearance of degenerating nerve debris and that this role is at least in part played by Schwann cells. Our evidence suggests that this $CCR2^-$ phagocyte may be sufficient for debris clearance in the sciatic nerve, and we propose that CCR2+ macrophages may play a different and less well-known role in Wallerian degeneration.

Disclosures: J. Lindborg: None. **R. Zigmond:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.18/A56

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant DK097223

Title: Reduced induction of injury-induced cytokines and neuropeptides after nerve injury in a mouse model of type 1 diabetes

Authors: *A. R. FILOUS, J. P. NIEMI, A. DEFRANCESCO-LISOWITZ, R. E. ZIGMOND; Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Neuropathy is a major complication for those suffering from diabetes. It is often associated with numbness, intractable pain, and loss of function. The exact mechanisms underlying diabetic neuropathy are not well-understood, but are believed to partially result from regenerative deficits that accumulate over time, leading to loss of innervation. Previous work from our lab has established that members of the gp130 family of cytokines (or neuropoietic cytokines) are induced after injury and play a role in signaling for regeneration in normal animals (Hyatt Sachs et al., 2010). Here, we explored the possibility that these cytokines may have a reduced induction after injury in diabetic mice, leading to nerve regeneration deficits. We used streptozotocin (STZ) to selectively deplete pancreatic β-islet cells, depleting insulin and ultimately leading to chronic hyperglycemia in these mice. This is the most commonly used chemically-induced model of type 1 diabetes. We injected mice with either multiple low dose

injections of STZ (5x 60mg/kg) or a single high dose injection (1x 200mg/kg) and examined the mice at least four weeks later. At this time, we superimposed nerve injuries [axotomy of the neurons in the superior cervical ganglion (SCG) and axotomy of the sciatic nerve] to study the regenerative response in diabetic mice compared to age-matched controls. Using both the SCG and dorsal root ganglia (DRG), we were able to examine the effects on both sympathetic and sensory neurons. We found that both ganglia have reduced induction of two gp130 cytokines, leukemia inhibitory factor and interleukin-6, after injury. This is accompanied by reduced phosphorylation of signal transducer and activator of transcription 3 (STAT3), a downstream effector of the gp130 signaling pathway. We also found various gp130-dependent regeneration-associated genes (Habecker et al., 2009) including galanin, vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide have reduced induction after injury as well. These changes may lead to deficits in neurite outgrowth of dissociated SCG and DRG neurons. Together, these data suggest a novel mechanism for diabetic neuropathy.

Disclosures: A.R. Filous: None. **J.P. Niemi:** None. **A. DeFrancesco-Lisowitz:** None. **R.E. Zigmond:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.19/A57

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant DK097223

NIH Grant NS077888

NIH Grant EY11373

Title: Overexpression of CCL2 in dorsal root ganglia is sufficient for enhanced axonal regeneration

Authors: *J. P. NIEMI, A. DEFRANCESCO-LISOWITZ, M. HOWARTH, R. ZIGMOND; Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Neuroinflammation plays a critical role in the regeneration of peripheral nerves following axotomy. An injury to the sciatic nerve leads to significant macrophage accumulation in the L5 DRG, an effect not seen when the dorsal root is injured. Recent evidence showed that macrophage accumulation around axotomized cell bodies is necessary for a peripheral conditioning lesion response. In response to an axonal injury, DRG neurons upregulate and release CCL2, a macrophage chemokine which acts on the receptor CCR2. In a CCR2 knockout mouse (CCR2-/-), CD11b+ macrophage accumulation was inhibited in the distal sciatic nerve

and in the axotomized DRG after injury. To ascertain the effect on regeneration, DRGs were placed in explant culture 1 week after a conditioning lesion. Increased outgrowth was seen in previously lesioned DRGs from WT but not CCR2-/- mice. These data suggest a relationship between macrophage accumulation near neuronal cell bodies and the regenerative capacity of neurons as well as highlighting the role CCL2/CCR2 signaling plays in mediating macrophage entry into DRGs after injury. To probe the importance of this signaling, we asked whether overexpression of CCL2 specifically in DRG neurons of uninjured mice would be sufficient to cause macrophage entry and enhanced regeneration, or whether other injury-derived signals are necessary. We found that CCL2 could be significantly overexpressed in DRG neurons by utilizing an adeno-associated virus (AAV) delivered intrathecally. AAV-CCL2 injection led to a time dependent increase in CCL2 mRNA expression compared to AAV-YFP controls, with a maximal response seen at 3 weeks post-injection. Consequently, CCL2 overexpression also led to a time dependent increase in CD11b+ macrophages in the L5 DRG compared to YFP controls, with a maximal response at 3 weeks post-injection, a time point used in all subsequent experiments. CCL2 overexpression and subsequent macrophage accumulation led to a conditioning-like increase in neurite outgrowth of DRG explants from mice injected with AAV-CCL2 compared to AAV-YFP controls. This increase in regeneration was dependent upon CCL2 acting through its primary receptor CCR2. When CCL2 was overexpressed in CCR2-/- mice, macrophage accumulation and enhanced regeneration were not seen. Finally, administration of AAV-CCL2 resulted in increased LIF mRNA and increased neuronal pSTAT3 in L5 DRG. Thus, CCL2 overexpression is sufficient to cause increased macrophage accumulation and increased regenerative capacity of DRG neurons. This likely occurs through the stimulation of signaling pathways critical for regeneration including the activation of the JAK-STAT pathway.

Disclosures: J.P. Niemi: None. **A. DeFrancesco-Lisowitz:** None. **M. Howarth:** None. **R. Zigmond:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.20/A58

Topic: A.07. Transplantation and Regeneration

Support: NIH (NCATS) CTSA TL1 TR000066

Title: Potential therapeutic applications of chondroitinase C for *in vivo* selective CSPG degradation

Authors: *J. B. GRAHAM, D. MUIR;
Univ. of Florida Col. of Med., Gainesville, FL

Abstract: Chondroitin sulfate proteoglycans (CSPGs) are potent inhibitors of axon regeneration in both the central and peripheral nervous system. CSPGs are constitutively expressed throughout the extracellular matrix and are postulated to maintain homeostasis of inherently sprouting nerve cell appendages through negative interactions of the glycosaminoglycan (GAG) chains. The formation of CSPG rich perineuronal nets that appear during maturation of spinal motor neuron pools are believed to suppress neuronal plasticity through this mechanism. Several laboratories have investigated the application of chondroitinase ABC (ChABC) to degrade the inhibitory CSPG GAG chains of perineuronal nets and induce neuronal plasticity with much success but recent studies within the peripheral nervous system have provided evidence that excessive sprouting may lead to aberrant regeneration. We have successfully enhanced peripheral axon regeneration *in vitro* through selective degradation of CSPG GAG chains using chondroitinase C (ChC), an isozyme of ChABC which is more selective towards chondroitin sulfate C than chondroitin sulfate A or dermatan sulfate. Selective degradation was supported by Alcian Blue and Coomassie Blue stained SDS-PAGE showing complete or partial removal of GAG chains and molecular weight shifts of CSPG core proteins compared to ChABC treatment. Additionally, western analysis confirmed selective degradation with neoepitope labeling of CSPG core proteins at different molecular weight shifts. Furthermore, western analysis has provided evidence that ChC does not degrade GAG chains associated with certain CSPG core proteins. Finally, we applied ChC or ChABC to fixed frozen sections of normal rabbit spinal cord and adult mouse brain and provided evidence that both enzymes have the ability to degrade GAG chains associated with perineuronal nets by producing neoepitope labeling immunoreactive towards C6S antibody. Although the enzymatic activity of ChC has been documented to be inhibited at physiological conditions, we have provided evidence of the contrary by producing C6S immunoreactive neoepitope following microinjection of ChC into the mouse sciatic nerve. Here we provide evidence of potential functional consequences following application of ChC *in vitro* and *in vivo*.

Disclosures: **J.B. Graham:** None. **D. Muir:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.21/A59

Topic: A.07. Transplantation and Regeneration

Support: NRF(BK21+) Grant 10Z201312372

KHIDI Grant HI14C0522

NRF Grant 2009-0093829

Title: Mesoporous silica nanoparticles as a delivery system for nerve regeneration

Authors: ***M. KIM**[1,2,3], H.-S. AHN[2,3], J.-W. KIM[2,3], H.-W. KIM[2,3,4], Y.-J. SON[6,7], J. HYUN[2,3,5]; [2]Inst. of Tissue Regeneration Engin., [3]Dept. of Nanobiomedical Sci. and BK21 PLUS NBM Global Res. Ctr. for Regenerative Medic, [4]Dept. of Biomaterial Science, Sch. of Dent., [5]Dept. of Rehabil. Medicine, Col. of Med., [1]Dankook Univ., Cheonan, Korea, Republic of; [6]Shriners Hosp. Pediatric Res. Ctr., [7]Dept. of Anat. and Cell Biol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Mesoporous silica nanoparticle (MSN) has many advantages as an efficient drug delivery system including a large surface area, high porosity and intrinsic biocompatibility which allow the absorption and release of multiple drugs and biomolecules. We aim to delineate whether PTEN inhibitor-conjugated MSN is more effective on the axonal outgrowth of peripheral nerve than the application of PTEN inhibitor without a delivery system *in vitro* condition and MSN can be loaded into various neurons and glial cells *in vivo* conditions. For *in vitro* study, bisperoxovanadium (BpV) (HOpic) was conjugated to functionalized and rhodamine-labeled MSNs (MSN-BpV) and then applied to primary cultured dorsal root ganglion (DRG) which were dissected from Sprague Dawley (SD) rats. We found that the efficiency of MSN transfection into DRGs were over 95% when the concentration of MSN within culture media was over 20μg/ml. After the application of MSN-BpV to DRGs, the maximal length of outgrowing DRG axons was longer than those only treated with BpV at 20 and 40μg/ml. Western blotting revealed that MSN-BpV treated DRGs showed lower pPTEN/PTEN level and lower pAkt/Akt level than those only treated with BpV at the same concentration and non-treated controls. MSNs were also directly injected into the brain, spinal cord, and DRGs of neonatal or adult SD rats. We found that MSNs were successfully internalized into the cytoplasm of neurons, astrocytes, oligodendrocytes and macrophages. We conclude that MSNs effectively deliver PTEN inhibitor into sensory neurons and enhance axonal outgrowth without cell toxicity *in vitro* and may play an important role in transport of drugs or biomolecules into neurons and glial cells in the central and peripheral nervous systems *in vivo*.

Disclosures: **M. Kim:** None. **H. Ahn:** None. **J. Kim:** None. **H. Kim:** None. **Y. Son:** None. **J. Hyun:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.22/A60

Topic: A.07. Transplantation and Regeneration

Support: SHRF Grant 2803

CIHR Grant MOP74747

MSSC Grant 2362

U of S COM Scholarship

Title: Brief electrical stimulation promotes immune cell clearance and polarizes macrophages toward a pro-repair M2 phenotype in focally demyelinated peripheral nerve

Authors: *N. A. MCLEAN[1,2], V. M. K. VERGE[1,2];
[1]Anat. and Cell Biol., Univ. of Saskatchewan, Saskatoon, SK, Canada; [2]CMSNRC, Saskatoon, SK, Canada

Abstract: Demyelinating diseases are characterized by segmental demyelination of axons and infiltration by cells of the monocyte lineage. We and others have shown that brief electrical stimulation (ES) at the time of peripheral nerve repair enhances regeneration of sensory and motor neurons including axonal remyelination. Recently we have shown that brief ES, 5 days after a focal demyelinating lesion, accelerates removal of myelin debris and myelin repair, promotes axonal protection and is accompanied by the enhanced clearance of ED-1 positive macrophages from the demyelination zone by 5 days post-ES. Macrophages in response to local microenvironment cues can alter their functional polarity, exhibiting either a pro-inflammatory (M1) or a pro-repair (M2) phenotype, with the latter important for the resolution of inflammation and tissue repair. Because ES dramatically impacts remyelination, including macrophage responses, we posited that the ES may be polarizing macrophages toward a pro-repair state. To examine this, the right tibial nerve of adult male Wistar rats was focally demyelinated by 1% lysophosphatidyl choline (LPC) injection just distal to the trifurcation of the sciatic nerve. Five days later, half of the animals were subjected to 1 hour continuous 20 Hz ES proximal to the injection site. At 5, 8, and 10 days post-LPC, animals were perfused and the right and left sciatic nerves removed and processed immunohistochemically to detect the impact of ES on macrophage phenotype. There is robust infiltration of ED-1 positive macrophages into the lesion site, the majority of which are also positive for the M1 phenotype associated markers TNF-α and iNOS by 5 days post LPC. By 8 and 10 days post-LPC (3 and 5 days post-ES) stimulated nerves displayed a significant reduction in the number of M1 phenotype macrophages, coupled with an increase in the percentage of ED-1 positive macrophages expressing the M2 phenotype markers CD206 and Arginase-1. This transition occurs at a time point coincident with an overall reduction in the numbers of ED-1 positive macrophages present within the lesion site. These results suggest that ES may help create an environment permissive for early remyelination by promoting a switch to a pro-repair M2 macrophage phenotype.

Disclosures: N.A. McLean: None. **V.M.K. Verge:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.23/A61

Topic: A.07. Transplantation and Regeneration

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 12134101)

Title: Lycium barbarum polysaccharide enhances the intrinsic growth capacity of dorsal root ganglion neurons

Authors: *N. P. B. AU[1], G. KUMAR[1], R. C. C. CHANG[3,5], K. F. SO[4,5], C. H. E. MA[1,2]; [1]Dept. of Biomed. Sci., [2]Ctr. for Biosystems, Neuroscience, and Nanotechnology, City Univ. of Hong Kong, Kowloon, Hong Kong; [3]Lab. of Neurodegenerative Diseases, Dept. of Anat., [4]Dept. of Ophthalmology, LKS Fac. of Medicine, The Univ. of Hong Kong, Pokfulam, Hong Kong; [5]State Key Lab. of Brain and Cognitive Sci., The Univ. of Hong Kong, Pokfulam, Hong Kong

Abstract: Unlike the central nervous system (CNS), the peripheral nervous system (PNS) can regenerate at a slow rate of axonal regrowth (1-2mm/day). Patients with proximal peripheral nerve injury such as brachial plexus injury often result in poor functional recovery largely due to the long distance axonal regrowth. Wolfberry (*Lycium barbarum*) is a well-known traditional Chinese medicine serves as an upper class herb without any known toxicity or side effect. Recent studies showed that one of its major active components *Lycium barbarum* polysaccharides (LBP) exhibit a wide range of beneficial activities including anti-aging, modulation of immune function, protection against liver damage and reduction in blood glucose levels in diabetes. In the CNS, LBP have been shown to promote the survival of retinal ganglion cells in an animal model of glaucoma, and reduce the Aβ-induced neurotoxicity in cortical neurons. However, the beneficial effect of LBP on axonal regeneration in nervous system remains largely unknown. In the present studies, we aim to examine if LBP could enhance the regenerative capacity of injured DRG neurons purified from the adult PNS. The dissociated primary DRG neuron is widely used to assess the intrinsic growth capacity and in gene expression profile studies. Our pilot data showed that LBP induced a marked increase in neurite outgrowth of DRG neurons at all concentrations we tested (0.1-300μg/ml) without any detectable cell survival effect. These data prompted us to examine the promoting effect of LBP on functional recovery after peripheral nerve injury. Further gene expression profile studies will provide insight into the molecular mechanism of LBP in PNS regeneration.

Disclosures: **N.P.B. Au:** None. **G. Kumar:** None. **R.C.C. Chang:** None. **K.F. So:** None. **C.H.E. Ma:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.24/A62

Topic: A.07. Transplantation and Regeneration

Support: Early Career Scheme (ECS) Grant, University Grants Committee, Hong Kong Special Administrative Region Government (Ref. No.: CityU 161212)

Title: A comparative proteomics analysis of proteins involved in modulating axonal growth in peripheral neurons

Authors: *J. S. VONG, C. H. E. MA;
Dept. of Biomed. Sci., City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Our previous study showed that the time window for the regenerating axons to re-innervate their target motor endplates was limited to 35 days in mice. We have established an animal model to study critical period by performing repeated sciatic crushes to confine the time in which regenerating axons from reaching the muscle within the critical period (short-crush, <35 days) or outside the critical period (long-crush, >35 days) where functional recovery is limited. Sciatic nerve was crushed 4 times at 2-day intervals (short-crush) and 4 times at 9-day intervals (long-crush). Plantar muscle contralateral and ipsilateral to the sciatic nerve crush was dissected out for proteomic study. Differential protein expression profile after short- and long-crush was resolved by second-dimension gel electrophoresis. Fifteen proteins (spots) were identified and they are differentially regulated after short- and long-crush. We also examined the protein expression profile after chronic muscle denervation. We identified one cytoskeleton protein which was highly upregulated not only in the muscle but also in dorsal root ganglion (DRG) neurons after peripheral nerve injury. We showed that preconditioned primary DRG neurons highly expressed this cytoskeleton protein and its up-regulation result in longer neurite outgrowth. Loss of function assay by siRNA in preconditioned DRG neurons showed a significant reduction in neurite length. Further loss-of-function assay in animals will be performed to assess functional recovery after peripheral nerve injury. **Theme and Topic:** A.07.b. Regeneration: PNS **Keywords**: PERIPHERAL NERVOUS SYSTEM, SCIATIC NERVE INJURY, DORSAL ROOT GANGLION, AXONAL GROWTH, PROTEOMICS

Disclosures: J.S. Vong: None. **C.H.E. Ma:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.25/A63

Topic: A.07. Transplantation and Regeneration

Support: CIHR

AIHS

endMS Alberta

Title: Adult Skin-derived precursor Schwann cells (aSKP-SCs), like acutely injured nerves Schwann cells, exhibit superior myelination and regeneration supportive properties compared to chronically denervated nerve Schwann cells

Authors: *R. KUMAR[1], S. SINHA[2], E. RAHARJO[1], A. HAGNER[1], M. STYKEL[1], K. SINGH[3], R. MIDHA[1], J. BIERNASKIE[1];
[1]Univ. of Calgary, Calgary, AB, Canada; [2]Univ. of Toronto, Toronto, ON, Canada; [3]McMaster Univ., Hamilton, ON, Canada

Abstract: Despite the innate capacity of peripheral nervous system to regenerate, severe nerve injury patients with chronic nerve denervation never regain full function. Within chronically injured nerve, Schwann cells (SCs) fail to maintain regeneration supporting phenotype, which leads to poor functional recovery in afflicted patients. The goal of this study is to search and evaluate an ideal SC population and provide improved autologous cell transplantation therapy to augment nerve repair. Skin derived precursors (SKPs) are multipotent stem cells, and show phenotypic similarities to neural crest cells. Neonatal SKPs are capable of differentiation into Schwann cells (SCs), which express multiple markers of immature myelinating SCs. Upon transplantation into dysmyelinated or injured peripheral nerves neonatal SKP-SCs not only are capable of survival but also exhibit remarkable myelination. In acute and delayed nerve repair models of severe injuries neonatal SKP-SCs are able to show robust remyelination and restore functional recovery in an array of behavioral tests. We further assessed the potential of adult SKP-SCs (aSKP-SCs) and tested their capacities for promyelinating transcription factor expression, proliferation, neurite outgrowth, and remyelination. Here we show in line with previous literature, following chronic denervation SCs lose the capacity to support axon regeneration and show less robust myelination. We hypothesize that recapitulating the early denervation phenotype; transplanting aSKPSCs in the chronically denervated nerve may restore remyelination and regeneration support capacity. We compared SCs within acute and chronic injured nerves and found that acutely injured nerve SCs exhibit signature marker expression (Oct-6 and Krox-20) of myelinating embryonic SCs during development. Our data suggest that chronically denervated nerve SCs fail to exhibit comparable myelinating phenotype, exhibit retarded proliferation, show diminished neurite outgrowth, and demonstrate inferior *in vitro*/*in vivo* myelination as compared to acutely injured nerve SCs. It is remarkable that aSKP-SCs express the markers of promyelinating SCs such as Sox-10, Oct-6 and Krox-20 and exhibit superior proliferation, neurite outgrowth, and *in vitro*/*in vivo* myelination, which is very similar to acutely or embryonic nerve SCs and superior to chronically denervated nerve SCs. Based on our findings we conclude that aSKP-SCs may serve as a potential source of myelinating SCs to repair injured nerves and can serve as a potential source of autologous cell therapy in patients.

Disclosures: **R. Kumar:** None. **S. Sinha:** None. **E. Raharjo:** None. **A. Hagner:** None. **M. Stykel:** None. **K. Singh:** None. **R. Midha:** None. **J. Biernaskie:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.26/A64

Topic: A.07. Transplantation and Regeneration

Support: SHC85600

Title: The axonal palmitoyl acyltransferases DHHC5 and DHHC8 are essential for retrograde injury signaling by the gp130/JAK/STAT3 pathway

Authors: *A. MONTERSINO, K. M. COLLURA, S. M. HOLLAND, G. M. THOMAS; Shriners Hosp. Pediatric Res. Ctr., Temple Univ., Philadelphia, PA

Abstract: The protein-lipid modification palmitoylation is an important regulator of protein targeting and trafficking. Interestingly, genetic mutation or loss of a number of palmitoyl acyltransferases (PATs, which catalyze palmitoylation) cause predominantly neuropathological defects, suggesting that palmitoylation plays particularly important roles in neurons. Although there have been several recent advances in the identification of roles and substrates of specific PATs, virtually nothing is known about PAT expression and distribution in peripheral neurons, and the roles of PATs in axons are largely unclear. We therefore sought to identify roles for specific PATs in peripheral axons. One process that is particularly important in peripheral axons is retrograde signaling after a nerve injury, in which proteins are physically transported from distal sites of damage to activate pro-regenerative transcription. One key retrograde signaling pathway involves the transmembrane receptor gp130, its associated kinase JAK, and JAK's substrate STAT3. Here, we report that gp130 is palmitoylated in transfected heterologous cells and endogenously in neurons. To identify potential regulators of gp130 palmitoylation we determined the distribution of all 23 mammalian PATs in sensory neurons. Strikingly, we found that only 2 of 23 PATs, DHHC5 and DHHC8, are enriched in sensory axons and that knockdown of these axonal PATs reduces both palmitoylation and surface targeting of gp130 in sensory neurons. Moreover, consistent with this impaired gp130 targeting, DHHC5 and DHHC8 are essential for retrograde signaling by gp130/JAK/STAT3. Together these findings identify the first axonally enriched PATs and the first axonal substrate for DHHC5/8. We will further discuss how these findings provide new insights into how injury response signals are coordinated and conveyed.

Disclosures: A. Montersino: None. **K.M. Collura:** None. **S.M. Holland:** None. **G.M. Thomas:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.27/A65

Topic: A.07. Transplantation and Regeneration

Support: NIH Grant 1R01DC012829

Title: Chemotherapeutics and the taste system: Using cyclophosphamide to study system recovery

Authors: *D. HARRIS[1], D. E. MORGAN[1], E. R. DELAY[2];
[2]Biol., [1]Univ. of Vermont, Burlington, VT

Abstract: Patients undergoing chemotherapy often report disturbances in their ability to taste. These deficits can lead to decreased appetite, malnutrition, and ultimately poorer prognoses. Previous work in this lab has examined the effects of the chemotherapeutic cyclophosphamide (CYP) on the taste system of mice. Initial analyses have shown biphasic (post-injection days 2-3, 9-12) deficits in taste sensitivity and acuity through behavioral measures. These behavioral measures directed immunohistochemical (IHC) assays examining the state of tissue at days 0, 4, 7, 10, 12, 16, and 21 following a single moderate dose of CYP (75 mg/kg). Briefly, mice were perfused using 4% paraformaldehyde, and the tongue was extracted and cryoprotected in 30% sucrose. After being frozen, slides were sectioned and stained for cell type (PLCβ2, SNAP25), proliferation (Ki67, BrdU), and cell death (TUNEL assay and caspase-3). Fungiform papillae showed (1) decreases in Ki67, PLCβ2, and BrdU expression 4 days post injection, (2) maintained deficits in PLCβ2 expression at 7 days post injection, and (3) increased Ki67 expression 10 days post injection. Circumvallate papillae showed decreased expression of (1) Ki67 at day 4 post injection, (2) PLCβ2 at 7 days post injection, and (3) PLCβ2 and BrdU at 10 days post injection. We are expanding upon these initial findings to more fully understand the extent to which the taste system is compromised by CYP. To do this, we are analyzing taste cell populations all days between the time points studied previously. This time-course will be critical for evaluating potential mechanisms underlying recovery of taste function. We are also looking at potential mechanisms involved in the restoration of these taste cell populations. Sox2, for example, is necessary for embryological development of the anterior tongue and taste system and may play a critical role in the recovery of the taste system following injury by CYP. Adult Sox2 expression in the taste system appears to decrease 4 days following CYP injection. Understanding the nature and mechanisms of tissue recovery can provide means to help those undergoing chemotherapy to have better nutritional intake and quality of life.

Disclosures: D. Harris: None. **D.E. Morgan:** None. **E.R. Delay:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.28/A66

Topic: A.07. Transplantation and Regeneration

Support: NIH NCRR 5P20RR016463-12

NIH NIGMS 5P20RR016463-12

Bates Student Research Fund

NSF DBI-1428210

Title: Sensory neuron involvement in caudal fin regeneration in zebrafish, Danio rerio

Authors: M. YOUNISS[1], *N. W. KLECKNER[2];
[1]Biol., [2]Biology/Neuroscience, Bates Col., Lewiston, ME

Abstract: Most organisms have the ability to regenerate certain body parts. However, some vertebrates have a particularly extensive regenerative capacity. For example, some amphibian limbs and fish tails can be regrown when lost, and both of these processes are well characterized. Amphibian limb regeneration has been found to be dependent on the presence of nerves; once denervated, a limb loses its ability to regenerate following an amputation. This dependence is due to the release of anterior gradient proteins (nAg, in salamanders) by Schwann cells. While zebrafish (*Danio rerio)* tail regeneration is phenotypically well characterized, neither its dependence on the presence of nerves nor its expression of anterior gradient proteins (*agr1 or 2* in zebrafish*)* during the process have been explored. This study provides the necessary preliminary experiments to ultimately compare amphibian limb and zebrafish caudal fin regeneration. We explored *agr2* expression in the caudal fin during normal regeneration in juvenile zebrafish (4-6 weeks old, chosen to ensure comparisons in later live imaging experiments). Additionally, multiple denervation techniques, including mechanical denervation and denervation by exposure to metronidazole or paclitaxel, were explored. Denervation was verified by comparing anti-acetylated tubulin antibody labeling of caudal fin axons of treated and control fish. We found that the expression of *agr2*was upregulated in the caudal fin during regeneration, although not specifically in the blastema. Additionally, while mechanical denervations did not consistently remove neural input, exposure of fish to 1 and 10 mM metronidazole in system water and intraperitoneal injection of 10 μM paclitaxel were promising methods for caudal fin axon degeneration. These findings can be used in later studies to determine if regeneration is dependent on neural input, as seen in amphibians. Denervation techniques will be utilized prior to amputation, and regeneration and *agr2* expression will be compared to normal phenotypic regrowth and expression. Conserved or contrasting pathways may highlight why most vertebrates have such a limited ability to regenerate.

Disclosures: M. Youniss: None. **N.W. Kleckner:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.29/A67

Topic: A.07. Transplantation and Regeneration

Support: Ochsner Clinic Foundation

Title: Transforming growth factor beta regulates expression of fibroblast growth factor -7 in schwann cells

Authors: D. H. NGUYEN[1], A. MUHAMMAD[2], *I. IWUCHUKWU[3], W. O. SULAIMAN[2]; [1]Neurosurg., [2]Ochsner Med. Ctr., New Orleans, LA; [3]NEUROLOGY, Ochsner Clin. Fndn., New Orleans, LA

Abstract: Background and Objective: Our previous works using a chronic denervation and axotomy rat model have demonstrated that transforming growth factor -beta (TGF-β1) and forskolin reactivated Schwann cells (SCs). We also found that expression of fibroblast growth factor-7 (FGF-7) decreased 3.8-fold. In this study, we examined the molecular mechanism of TGF-β and forskolin on the expression of FGF-7 in cultured primary SCs. Methods: SCs were isolated from the sciatic nerve of adult female Sprague Dawley rat. SCs were used at passage 3-6. SCs (5 x10^5 cells) were cultured on poly-L-lysine coated 12-well tissue cluster for 6 days, starved overnight in DMEM, and stimulated with forskolin (0.25 or 0.5 uM), TGF-β (1 or 5ng/mL), or TGF-β plus forskolin for 24 hours. SCs were also pretreated with LY2109761 (0.5uM), a TGF-β receptor type I/II dual inhibitor, for 15 min prior to stimulation with TGF-β (1ng/mL) plus forskolin (0.5 uM) for 6 hours. Total RNA was isolated, reverse transcribed followed by real-time Taqman qPCR amplification. Cycle threshold (Ct) data were normalized to the ribosomal protein (RPLPO) reference gene and fold change relative to the untreated SCs were determined using the delta-delta Ct method. Results: At the concentrations of TGF-β and forskolin used in the *in vivo* studies, treatment of SCs with TGF-β (1ng/mL) alone or in combination with forskolin (0.5 uM) for 24 hours resulted in a 5.3-and 2.8 fold decrease in FGF-7 expression compared to untreated controls. No changes in FGF-7 expression were found with forskolin only treatment. Increasing the concentration of TGF-β (5 ng/mL) resulted in a 9.1 fold reduction in FGF-7 expression; whereas decreasing the concentration of forskolin (0.25 uM) resulted in a 2.2-fold reduction; the combined treatment resulted in a 1.4-fold increase in expression of FGF-7. Treatment with TGF-β/forskolin for 6 hours resulted in a 4.0-fold decrease in FGF-7 expresssion; Blocking of TGF-β receptor with LY2109761 lead to a decrease of 2.7-fold. Conclusion: We showed that expression of FGF-7 in SCs is regulated by TGF-β, and that addition of forskolin modulates TGF-β effect on FGF-7 expression. FGF-7 may have a role in nerve regeneration by affecting the responses of other cells, namely epithelial cells. Hence,

modulation of FGF-7 expression in SCs may be necessary in chronic nerve repair and regeneration.

Disclosures: D.H. Nguyen: None. **A. Muhammad:** None. **I. Iwuchukwu:** None. **W.O. Sulaiman:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.30/A68

Topic: A.07. Transplantation and Regeneration

Support: NIH NIDCD 1R01DC012829

Title: Cyclophosphamide-induced loss in murine olfactory systems

Authors: N. AWADALLAH[1], K. R. PROCTOR[2], E. R. DELAY[3], *R. J. DELAY[3];
[1]Neurosci. Undergraduate Program, [2]Med. Lab. Sci., [3]Biol., Univ. of Vermont, Burlington, VT

Abstract: Patients undergoing chemotherapy often report profound changes in their ability to taste and smell during and after drug therapy. Cyclophosphamide (CYP), one of the first chemotherapy agents, is an alkylating agent known to disrupt taste functions by its cytotoxic effects on taste buds and by temporarily halting the adult taste cell renewal process. However, little is known about the effects of CYP on the olfactory system. Since the sense of smell depends on the presence of olfactory neurons that undergo replacement similar to the taste system, we asked if a single injection of CYP would affect olfactory neurons? We examined the effects of CYP on olfactory neurons of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). We used an antibody to Ki67, a protein only expressed in cells undergoing division (G1, S, G2, mitosis). Male C57BL/6J mice were given a single IP injection of CYP (75 mg/kg) or saline and sacrificed from 1 day to 80 days post-injection. Mice were perfused with 4% paraformaldehyde, decalcified with EDTA, cryto-protected, sectioned and incubated with Ki67 antibody (Thermo scientific). There were clear differences between the MOE and the VNO across all the time points. At 1 day post injection the MOE looked damaged, especially in the dendritic region while the VNO was structurally unaffected. However both tissues showed a decrease in KI67 protein label compared to controls. By day 2, neither tissue showed any Ki67 labeling. In addition both the VNO and the MOE showed significant drops in Ki67 labeling 14 days post injection and 60 days post injection compared to controls. So far our data suggest that the MOE was more affected by CYP than the VNO. These findings provide important insights into the loss of olfactory function reported by patients during and after chemotherapy treatment.

Disclosures: N. Awadallah: None. **K.R. Proctor:** None. **E.R. Delay:** None. **R.J. Delay:** None.

Poster

291. Peripheral Nervous System Regeneration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 291.31/A69

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NRF Grant 2012R1A2A2A01046132

Title: Zinc supplementation by zinc plus cyclo-(His-Pro) increases progenitor cell proliferation after hypoglycemia

Authors: *A. KHO[1], J. KIM[1], I. KIM[1], B. CHOI[1], S. LEE[2], M. SOHN[3], S. SUH[1];
[1]Dept. of physiology, [2]Dept. of neurology, Hallym Univ., Chuncheon, Korea, Republic of; [3]Dept. of nursing, Inha university, Incheon, Korea, Republic of

Abstract: Neurogenesis after brain injury contributes to neuronal replacement and functional recovery. Hypoglycemia-induced brain injury increases transient neurogenesis in the dentate gyrus (DG) subgranular zone (SGZ). At four weeks after severe hypoglycemia, progenitor cell proliferation in the SGZ was reduced below baseline in sham-operated rats. In our previous study, zinc chelation or vesicular zinc depletion reduced hypoglycemia-induced neurogenesis. To test whether zinc supplementation can increase hippocampal neurogenesis after severe hypoglycemia we injected zinc with histidine and proline compounds (Cyclo-(His-Pro)) for 3 weeks starting at one week after hypoglycemia. It is well known that histidine-proline-rich glycoprotein plays a role in zinc transport. Cyclo-(His-Pro)(CHP) is a naturally occurring peptide found in the central nervous system (CNS) that can affect brain function after systemic administration. CHP has been used as a dietary supplement and is known to be a neuromodulator capable of crossing the blood-brain barrier (BBB). Several studies found that CHP improves zinc absorption and maintains steady concentrations of zinc for more than 12 hours. To investigate whether zinc supplementation can improve hypoglycemia-induced neurogenesis we used an animal model of insulin-induced hypoglycemia. Acute hypoglycemia was induced by intraperitoneal injection of human insulin (10 U/kg), and then iso-electricity was maintained for 30 minutes. We examined the neurogenic effects of zinc supplementation with zinc plus cyclo-(His-Pro) at 4 weeks after hypoglycemia in the SGZ. Sham-operated rats were also injected with or without zinc plus cyclo-(His-Pro) for 3 weeks. We performed immunohistochemistry using a ki67 antibody to detect progenitor cell proliferation. At four weeks after hypoglycemia, the number of Ki67 (+) cells decreased below baseline compared to sham-operated rats. However, three weeks supplementation of zinc with cyclo-(His-Pro) increased the number of Ki67 (+) cells in the hippocampal SGZ above baseline in hypoglycemia experienced rats. Furthermore, even in sham-operated rats the number of Ki67 (+) cells were higher than baseline levels The present study demonstrated that zinc plus cyclo-(His-Pro) supplementation positively affected hippocampal neurogenesis after severe hypoglycemia.

Disclosures: **A. Kho:** None. **J. Kim:** None. **I. Kim:** None. **B. Choi:** None. **S. Lee:** None. **M. Sohn:** None. **S. Suh:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.01/A70

Topic: B.02. Ligand-Gated Ion Channels

Support: Wings For Life

Title: A G protein-binding domain within the M3-M4 loop of the α7 nicotinic acetylcholine receptor enables a downstream calcium signaling response beyond the time course of channel activation

Authors: ***J. KING**, M.-K. LIN, N. KABBANI;
Krasnow Inst., Fairfax, VA

Abstract: α7 nicotinic acetylcholine receptors (nAChRs) play an important role in various aspects of synaptic transmission via their regulation of intracellular signaling pathways. In recent studies, we have demonstrated an important functional role for α7 nAChR interactions with intracellular heterotrimeric GTP binding proteins in various types of cells. Here we show that direct coupling of the intracellular loop of the α7 receptor to Gαq enables a downstream calcium signaling response that persists beyond the expected time course of channel activation. This process is made possible via an evolutionary preservation of a G protein-binding cluster (GPBC) within the M3-M4 loop of nAChRs. A specific mutation of the GPBC in α7 (α7-345-348A) abolishes interaction with G proteins while having no effect on the synthesis, α-bungarotoxin binding properties, or cell-surface trafficking of the receptor. Expression of α7-345-348A does however significantly attenuate the downstream calcium signaling response following activation of the receptor by the α7 selective agonist choline. This data provides novel evidence on the existence of a metabotropic G protein-binding domain within the α7 receptor and a mechanism for nAChR mediated signaling in cells.

Disclosures: **J. King:** None. **M. Lin:** None. **N. Kabbani:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.02/A71

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA035942

NIH Grant DA030396

NIH Grant GM103801

NIH Grant GM48677

Frederick N. Andrews Fellowship

John Davisson Endowment Research Award

Title: Removal of α4 nAChR subunits from adult VTA neurons alters VTA dopamine neuron excitability and locomotor activity

Authors: *S. E. ENGLE[1], J. N. BERRY[1], M. C. ARVIN[1], J. M. MCINTOSH[2,3], R. M. DRENAN[1];
[1]Medicinal Chem. and Mol. Pharmacol., Purdue Univ., West Lafayette, IN; [2]George E. Wahlen Veterans Affairs Med. Ctr., Salt Lake City, UT; [3]Psychiatry and Biol., Univ. of Utah, University of Utah, UT

Abstract: Nicotinic acetylcholine receptors (nAChRs) containing α4 and/or α6 subunits have a high sensitivity to nicotine and are implicated in nicotine's reinforcing properties. Determining if nicotine's effects are mediated by receptors containing both subunits, α4α6* nAChRs, remains challenging. In these experiments, we sought to study the effect of removing α4 subunits only from ventral tegmental area (VTA) neurons in adult mice. To do this, a viral vector directing expression of Cre recombinase was injected into the VTA of adult mice with loxP sites flanking the α4 subunit gene (α4flox mice). When α4 subunits are removed from the VTA of adult α4flox mice using Cre, nAChR function is diminished, as seen by significantly reduced ACh-evoked currents in VTA dopamine (DA) neurons. VTA DA neuron action potential firing is also modified in α4flox mice upon removal of α4 subunits from the VTA. Using current-clamp mode, we measured firing frequency during 2 sec current injection steps. When α4 subunits are removed, VTA DA neurons are more excitable. Small current injections (+20 pA) cause the firing rate of Cre(+) neurons to increase significantly compared to Cre(-) neurons. From these results we hypothesized that removal of α4* nAChRs from GABAergic neurons in VTA results in less tonic inhibition of VTA DA neurons. To test this we measured spontaneous inhibitory postsynaptic currents (IPSCs) on VTA DA neurons. Indeed, we saw that the frequency, but not amplitude, of IPSCs was significantly reduced when α4 subunits were removed from the VTA. To further test this hypothesis with a behavioral measure, we crossed α4flox mice with α6L9S mice, which express hypersensitive nAChRs containing α6 subunits. In α6L9S mice, low doses of nicotine stimulate locomotor activity via α4α6* nAChRs. Removing α4 subunits from the VTA of adult α6L9S mice using our Cre/loxP approach rendered mice insensitive to injected nicotine in locomotor activation assays (confirming removal of α4 using behavior), but increased spontaneous locomotor activity (consistent with disinhibition of DA neurons). ACh-evoked

currents in VTA DA neurons in α4floxα6L9S mice are also dramatically reduced when α4 subunits are removed from the VTA. Current experiments include assessing the ability of nicotine to alter synaptic plasticity in VTA (with AMPA/NMDA ratios) and nucleus accumbens (by measuring dendritic spine morphology) of adult mice where α4 has been removed from VTA. Overall, this study employing removal of VTA α4 subunits from the adult brain highlights the importance of both 1) α4* nAChRs in VTA GABAergic neurons, and 2) the substantial role played by α4 subunits in α6* nAChR function. Supported by NIH grant DA035942.

Disclosures: **S.E. Engle:** None. **J.N. Berry:** None. **M.C. Arvin:** None. **J.M. McIntosh:** None. **R.M. Drenan:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.03/A72

Topic: B.02. Ligand-Gated Ion Channels

Support: NARSAD Young Investigator Award

NIH Grant DA030396

Title: Nicotine dependence reveals distinct responses from neurons and their resident nicotinic receptors in medial habenula

Authors: P.-Y. SHIH[1], *R. M. DRENAN[2];
[2]Medicinal Chem. and Mol. Pharmacol., [1]Purdue Univ., West Lafayette, IN

Abstract: Nicotinic acetylcholine receptors (nAChRs) are the molecular target of nicotine. nAChRs in the medial habenula (MHb) have recently been shown to play a role in nicotine dependence, but it is not clear which nAChR subtypes or MHb neuron types are most important. To identify MHb nAChRs and/or cell types that play a role in nicotine dependence, we studied these receptors and cells with brain slice electrophysiology using both acute and chronic nicotine application. Cells in ventral MHb inferior (MHbVI) and lateral (MHbVL) subregions expressed functional nAChRs with different pharmacology. Further, application of nicotine to cells in these subregions led to different action potential firing patterns. The latter result was correlated with a differing ability of nicotine to induce nAChR desensitization. Chronic nicotine caused functional up-regulation of nAChRs selectively in MHbVI cells but did not change nAChR function in MHbVL. Importantly, firing responses were also differentially altered in these subregions following chronic nicotine. MHbVI neurons treated chronically with nicotine exhibited enhanced basal pacemaker firing but a blunted nicotine-induced firing response. MHbVL neurons did not change their firing properties in response to chronic nicotine. Together, these results suggest that acute and chronic nicotine differentially affect nAChR function and output of cells in MHb

subregions. Because the MHb extensively innervates the interpeduncular nucleus (IPN), an area critical for both affective and somatic signs of withdrawal, these results could reflect some of the neurophysiological changes thought to occur in the MHb to IPN circuit in human smokers.

Disclosures: P. shih: None. **R.M. Drenan:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.04/A73

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant R21 DA027070

NIH grant R21 DA036059

Barrow Neurological Foundation Start-up Funds

Title: Development and validation of an α3β4 nicotinic acetylcholine receptor (nAChR) high-throughput screening- (HTS-) ready assay

Authors: *P. WHITEAKER[1], M. KASSNER[2], B. EATON[1], J. PETIT[3], N. MEURICE[3], H. YIN[2];
[1]Div. Neurobiol, Barrow Neurolog. Inst., Phoenix, AZ; [2]Cancer and Cell Biol. Div., Translational Genomics Inst., Scottsdale, AZ; [3]Translational Chem. Biol. Lab., Mayo Clin., Scottsdale, AZ

Abstract: Genome-wide association studies (GWAS) have implicated α3β4*-nAChR in susceptibility to tobacco use and dependence, particularly among youth. The associated phenotypes include heavy smoking (daily cigarettes smoked), Fagerström Test for Nicotine Dependence scores, and age dependent severity of dependence. A link has also been established to lung cancer susceptibility. Animal models are consistent with a role for this subtype in nicotine dependence and aversive behavior. We generated α3β4-nAChR-expressing SH-EP1 cell lines using stable transfection with pcDNA3.1zeo and pcDNA3.1hygro vectors, followed by selection of resistant monoclones. Highly-functional monoclones were identified by measuring nAChR-mediated 86Rb+ efflux. One of the monoclones was selected for developing an (HTS)-ready 384-well format assay using FLIPR membrane potential sensing dye. The assay was optimized by fine-tuning several assay conditions including, but not limited to, cell seeding density, incubation time following cell seeding, plate type, dye dilution, dye incubation time, drug addition times and volumes, and data capture time. The optimized assay produced robust (Z' scores ≈0.8), reproducible (signal CV values in range of 4.3 - 7.6 %), nAChR mediated (could be blocked by the non-competitive antagonist mecamylamine) signals. This performance

exceeds that required for acceptance by NIH MLPCN (Z' >0.5 and CV mean signal + 3SD. An agonist/PAM hit rate of ≈1% was observed, with excellent run-to-run reproducibility (25 hits in common across 2 runs, 1 unique hit per run). In antagonist mode, in the presence of EC90 nicotine, hits were identified as < mean signal + 3SD. A total of 36 hits were identified (1.6% hit rate), also with excellent reproducibility. Using 86Rb+ efflux as an orthogonal assay, we confirmed 44 of the 62 total prospective hits identified across both the agonist and antagonist testing modes (a healthy 71% hit-confirmation / validation rate). Most compellingly, three structurally-related compounds were confirmed to have previously-unappreciated PAM activity on α3β4-nAChR. These data serve to validate the completed assay's suitability for use in HTS.

Disclosures: P. Whiteaker: None. **M. Kassner:** None. **B. Eaton:** None. **J. Petit:** None. **N. Meurice:** None. **H. Yin:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.05/A74

Topic: B.02. Ligand-Gated Ion Channels

Support: Ecole des Neurosciences de Paris

Fondation pour la Recherche sur le Cerveau/Rotary Club de France

Agence Nationale de la Recherche

Title: Nicotinic transmission onto layer 6 cortical neurons relies on synaptic activation of non-α7 receptors

Authors: Y. A. HAY, B. LAMBOLEZ, *L. TRICOIRE;
Univ. Pierre Et Marie Curie-CNRS-INSERM, Paris, France

Abstract: Nicotinic excitation in neocortex is mediated by low-affinity α7 receptors and by high-affinity α4β2 receptors. There is evidence that α7 receptors are synaptic, but it is unclear whether high-affinity receptors are activated by volume transmission or synaptic transmission. To address this issue, we characterized responses of excitatory layer 6 (L6) neurons to optogenetic release of acetylcholine (ACh) in cortical slices. L6 responses consisted in a slowly decaying α4β2 current and were devoid of α7 component. Evidence that these responses were mediated by synapses was 4-fold. 1) Channelrhodopsin-positive cholinergic varicosities made close appositions onto responsive neurons. 2) Inhibition of ACh degradation failed to alter onset kinetics and amplitude of currents. 3) Quasi-saturation of α4β2 receptors occurred upon ACh release. 4) Response kinetics were unchanged in low release probability conditions. Train stimulations increased amplitude and decay time of responses and these effects appeared to

involve recruitment of extrasynaptic receptors. Finally, we found that the α5 subunit, known to be associated with α4β2 in L6, regulates short-term plasticity at L6 synapses. Our results are consistent with previous anatomical observations of widespread cholinergic synapses and suggest that a significant proportion of these small synapses operate via high-affinity nicotinic receptors.

Disclosures: **Y.A. Hay:** None. **B. Lambolez:** None. **L. Tricoire:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.06/A75

Topic: B.02. Ligand-Gated Ion Channels

Support: NSERC Discovery Grant (CDCB)

Title: Functional characterization of α4β2* nicotinic receptors in principal neurons of the young postnatal mouse hippocampal formation

Authors: B. Y. T. CHUNG, *C. D. BAILEY;
Dept. of Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: The hippocampal formation plays an important role in learning, memory and attention. The normal development and mature function of this compound brain region depends on cholinergic neurotransmission mediated by the nicotinic class of acetylcholine receptor (nAChR). We have recently demonstrated in CD1-strain mice that the α4β2* isoform of nAChR mediates direct inward currents and facilitates excitation in principal (pyramidal) neurons of the hippocampal formation sub-region known as the cornu ammonis area 1 (CA1). This signaling is developmentally regulated with the greatest magnitude of α4β2* nAChR responses occurring during the first two weeks of postnatal life. As the hippocampal formation also comprises the cornu ammonis area 3 (CA3), dentate gyrus (DG), subiculum (SUB) and entorhinal cortex (EC), we sought in this current study to characterize α4β2* nAChR function within the principal neurons of these sub-regions during the first two weeks of mouse postnatal development. Whole-cell electrophysiological responses to acetylcholine (ACh) were recorded for visually-identified principal neurons of the CA1, CA3, DG, SUB and EC layer VI (EC-VI) within acute brain slices collected from male CD1 mice between postnatal days 5 and 10. Recordings were made in the presence of atropine to block muscarinic acetylcholine receptors and methyllycaconitine to block α7 subunit-containing nAChRs, in order to isolate pharmacologically α4β2* nAChR responses. We found that the magnitude of α4β2* nAChR responses varied across sub-regions of the hippocampal formation. Principal neurons in the SUB and EC-VI exhibited greater inward currents and a greater rise in membrane potential in response to α4β2* nAChR activation when

compared with neurons in the CA1, CA3 and DG. Within the hippocampus proper, principal neurons in the CA1 exhibited greater inward currents compared with both the CA3 and DG sub-regions. Interestingly, even though the magnitude of inward currents mediated by α4β2* nAChRs varied significantly across the hippocampal formation, we found no differences in the ability of ACh to accelerate action potential firing frequency among these sub-regions. Our findings demonstrate that functional α4β2* nAChRs are present in principal neurons of the hippocampal formation in young postnatal mice and that the characteristics of their responses to ACh vary across sub-regions of this compound brain region.

Disclosures: B.Y.T. Chung: None. **C.D. Bailey:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.07/A76

Topic: B.02. Ligand-Gated Ion Channels

Support: UAE University Grant

Title: Curcumin potentiates human α7-nicotinic acetylcholine receptors

Authors: *K.-H. S. YANG[1], S. M. NURULAIN[2], F. C. HOWARTH[3], M. OZ[2];
[1]Chapman Univ., Orange, CA; [2]Pharmacology, Fac. of Med. and Hlth. Sci., [3]Physiology, Fac. of Med. and Hlth. Sci., UAE Univ., Al Ain, United Arab Emirates

Abstract: Curcumin, a polyphenolic compound isolated from the rhizomes of Curcuma longa (turmeric), has been shown to exhibit a wide range of pharmacological activities including treatments of Alzheimer's disease, and cystic fibrosis, and inflammation. Although mechanisms of these effects are largely unknown, several types of voltage-gated ion channels and transporters have been suggested to be involved in mediating pharmacological actions of curcumin. However, the effects of curcumin on ligand-gated ion channels have not been described earlier. In this study we have investigated the effect of curcumin application on the functional properties of human α7-nicotinic acetylcholine (nACh) receptors. cRNA encoding for homomeric human α7-nicotinic acetylcholine (nACh) receptors were expressed in Xenopus oocytes. Ion currents mediated by the activation of nACh receptors were recorded using two-electrode voltage clamp method. Our results indicated that curcumin caused a significant potentiation of nACh receptor-mediated ion currents. The effect of curcumin (0.1 to 10 μM) was reversible and gradually reached a steady-state level within 10 min application time. Maximal amplitudes of currents activated by 100 μM ACh were significantly enhanced by curcumin in a reversible and concentration-dependent manner. In earlier studies, agonists of α7-nACh receptors have been shown to have therapeutic effects on Alzheimer's disease and inflammation. Therefore, our

results suggest that potentiation of α7-nACh receptors by curcumin can mediate some of its therapeutic actions in Alzheimer disease and inflammation.

Disclosures: **K.S. Yang:** None. **S.M. Nurulain:** None. **F.C. Howarth:** None. **M. Oz:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.08/A77

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant 1R21DA033831

NIH Grant 41DA032464-01

Howard Hughes Medical Institute Biosystems Dynamics Summer Institute

Howard Hughes Medical Institute Education Grant

Title: Investigating the role of the nicotinic receptor modulator, lynx2, on cholinergic-based anxiety mechanisms

Authors: *K. R. ANDERSON, H. WANG, J. MIWA;
Lehigh Univ., Bethlehem, PA

Abstract: Anxiety disorders are among the most prevalent of mental disorders in modern society. The anxiety response is a normal and advantageous reaction to a stressor but disorders can develop when individuals cannot return to baseline once the stressor has resolved. Current treatments for individuals suffering from anxiety disorders only temporality relive the symptoms without treating the cause, due to a lack of full understanding of the biological underpinnings of anxiety. Additionally, many anxiety sufferers self-medicate by smoking, suggesting a novel role of the cholinergic system in anxiety modulation. We are using a candidate gene approach to uncover these underpinnings, focusing on the role of a cholinergic modulator, lynx2, highly expressed in the basolateral aspect of the anxiety structure, the amygdala (BLA). Lynx2 proteins bind to and suppress cholinergic receptors (nAChRs). Consistent with its high expression, mice lacking lynx2 (lynx2KO), demonstrate elevated anxiety levels across several assays (light-dark, open-field, etc.). We hypothesize that experience-dependent plasticity in the amygdala plays a role in the return to baseline state, and that this is subject to cholinergic modulation. To address this we are performing behavioral pharmacological studies in lynx2KO mice to measure anxiety responses along with electrophysiology studies. Sensitivity to nicotine is augmented in lynx2KO mice in both the light/dark assay and slice physiology. Further investigations into the specific nAChR subtypes are being conducted with several specific inhibitors and uncovering a shift in receptor subtype without lynx2 presence, suggesting a role for lynx2 in receptor subunit

composition. Synaptic plasticity is altered in the BLA between wild-type and lynx2KO mice and normalcy could be restored by pharmacological manipulation of nAChRs. These data suggest that addressing synaptic plasticity may be a promising avenue by which to return individuals back to baseline states. To address this further, fear extinction is being used to assess modification of the fear response over time. Understanding of how amygdalar output can be altered by lynx and cholinergic pathways could help in the development of treatments for anxiety disorders such as PTSD.

Disclosures: K.R. Anderson: None. **H. Wang:** None. **J. Miwa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ophidion Inc..

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.09/A78

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS-SNRP U54NS039408

NCRR-RCMI Program G12RR03035

Title: Cembranoids structure-activity relationship for protection against diisopropylfluorophosphate damage

Authors: *V. A. ETEROVIC[1], M. CARRASCO[1], D. PEREZ[1], H. Y. EBRAHIM[2], P. A. FERCHMIN[1], K. A. EL SAYED[2];
[1]Biochem., Univ. Central Del Caribe, Bayamon, PR; [2]Dept. Basic Pharmaceut. Sci., Sch. of Pharm., Univ. of Louisiana, Monroe, LA

Abstract: Diisopropylfluorphosphate (DFP) is a toxic organophosphorous compound, which produces neurodegeneration. Post-application of the neuroprotective compound 4R-cembranoid (4R) ameliorates this damage. *In vitro*, the population spike (PS) of the hippocampal slice is decreased by DFP and recovered by post-application of 4R. The protective activity of 4R is triggered by the inhibition of the alpha7 nicotinic acetylcholine receptor (nAChR). A study of neuroprotection produced by fourteen 4R analogues - using the hippocampal slice model - produced a preliminary pharmacophore; which suggested that two hydrophobic regions in the cembranoids molecule are responsible for binding to the receptor and an electronegative atom forms a hydrogen bond with the receptor, which effects receptor inhibition. The purpose of the present work was to further characterize the structure-activity relationship between cembranoid molecular structure and their protective activity against DFP using the hippocampal slice preparation. Concentration-effect curves (nM - μM) were obtained for 21 cembranoids.

Seventeen analogues displayed standard curves, which were fitted to the four-parameters logistic equation. The most potent analogue had an EC50 of 0.6 nM and a maximum effect of 77% PS recovery; the least potent analogue had an EC50 of 2.3 μM and a maximum recovery of 120%; there was no correlation between the EC50 and maximum recovery values. These observations are consistent with the preliminary pharmacophore assumption that the binding and the inhibitory regions are located in different parts of the cembranoid structure. The four remaining analogues displayed a complex behavior; protective activity was seen at low and at high concentrations, but not at intermediate concentrations. Thus the ideal neuroprotective cembranoid must display high affinity and efficacy at the inhibitory site and low affinity for the positive modulator site.

Disclosures: **V.A. Eterovic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Universidad Central del Caribe. **M. Carrasco:** None. **D. Perez:** None. **H.Y. Ebrahim:** None. **P.A. Ferchmin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Universidad Central del Caribe. **K.A. El sayed:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ULM College of Pharmacy.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.10/A79

Topic: B.02. Ligand-Gated Ion Channels

Support: Agence Nationale de la Recherche (JCJC2014 Program)

NARSAD Young inverstigator grant from the Brain and Behavior Research Foundation

DIM Cerveau & Pensée, Région Île-de-France

Title: Optical control of midbrain dopamine neurons using a light-inhibited nicotinic receptor

Authors: *R. DURAND-DE CUTTOLI[1], F. MARTI[1], S. PONS[2], D. TRAUNER[3], R. H. KRAMER[4], U. MASKOS[2], P. FAURE[1], A. MOUROT[1];
[1]Neurosci. Paris-Seine lab, Univ. Pierre Et Marie Curie, Paris, France; [2]Dept. de Neurosciences, Inst. Pasteur, Paris, France; [3]Dept. of Chem., Ludwig-Maximilians-University (LMU), Munich, Germany; [4]Dept. of Mol. and Cell Biol., UC Berkeley, Berkeley, CA

Abstract: Dopamine (DA) neurons of the Ventral Tegmental Area (VTA) show two different firing patterns : a regular pattern associated with a tonic (constant and low) release of DA in the target structures; and a « burst » pattern associated with a phasic release of DA and involved in

reinforcement learning and reward prediction. Previous studies in the lab have identified the β2-containing nicotinic acetylcholine receptor (β2*nAChR) as a major player of the bursting activity : nicotine injection switches the activity of the DA neuron from tonic to phasic, while deletion of the β2 subunit results in DA neurons that only fire tonically and don't respond to nicotine. Assessing the precise role of the β2nAChR in the formation of bursts and in behavioral decision making processes requires methods for the acute, reversible control of this receptor subtype at time scales compatible with synaptic transmission. To this aim, we are developing new technologies for the optical control of specific nAChR subtypes *in vivo*. Notably, we have engineered a β2-containing, light-inhibited nAChR (β2*LinAChR). This designed receptor presents at its surface a free cysteine amino-acid for the anchoring of a chemical photoswitch. The photoswitch Maleimide-Azobenzene-HomoCholine (MAHoCh) is made of three components: a Maleimide group for bioconjugation to the cysteine mutant, an Azobenzene photoswitch that can be photoisomerized between its cis and trans states using violet and green light, respectively, and a Homo-Choline competitive antagonist. After covalent attachment of MAHoCh, β2LinAChRs respond normally to nicotine and acetylcholine, but can be rapidly antagonized using violet light (390 nm). Illumination with green light (520 nm) withdraw the homocholine group from its binding pocket, and restores the function of β2*nAChRs. We are using a lentiviral vector strategy to express β2*LinAChRs selectively in the VTA. Patch-clamp recordings of VTA DA neurons show that LinAChRs can be used to optically inhibit nicotinic inputs to the VTA. Furthermore, using *in vivo* extracellular recordings, we show that β2*LinAChR can be used to rapidly and reversibly modulate not only the spontaneous bursting activity of DA neurons, but also the bursts induced by intravenous injection of nicotine. Our findings establish a causal role for β2*nAChRs in regulating the excitability of VTA DA neurons.

Disclosures: R. Durand-De Cuttoli: None. **F. Marti:** None. **S. Pons:** None. **D. Trauner:** None. **R.H. Kramer:** None. **U. Maskos:** None. **P. Faure:** None. **A. Mourot:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.11/A80

Topic: B.02. Ligand-Gated Ion Channels

Title: Use of the novel toxin M2E11R to study the role of α6- nAChRs in visual function

Authors: D. BARLOSCIO[1], E. CERRI[1], L. DOMENICI[1], R. LONGHI[2], M. MORETTI[3], C. GOTTI[3], *N. ORIGLIA[1];
[1]CNR- Neurosci. Inst., PISA, Italy; [2]ICRM, [3]Neurosci. Inst., CNR, Milan, Italy

Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are highly expressed in the visual system. Increasing attention has been given to α6-containing nAChRs as they have a limited distribution in the brain but are highly and selectively expressed in the mesocorticolimbic and visual pathways. In particular, α6β2 nAChRs are present in the retinal ganglionic cell layer, in the optic nerve and in retina terminals but their physiological role is not well known. We have identified and characterized a family of toxins that are selective antagonists for the α6β2 receptors. We used one of these toxin (M2E11R, m.w.=1738), to investigate the impact of α6-containing nAChRs, on visual function in male Long-Evans rats. Visual function was assessed using flash/pattern (F-ERG and P-ERG) electroretinogram and cortical visual evoked potential (VEPs), after intraocular injection of M2E11R in one eye (1μM), using the vehicle injected contralateral eye as the control. We first used dark (scotopic) and light (photopic) adaptation to record F-ERG responses. Alternating gratings of different spatial frequencies and contrast were used to evoke VEPS and P-ERG. Moreover, the localization of α6-containing receptors in retinal tissue was performed using a fluorescently tagged M2E11R (Alexa Fluo 488) toxin. Our results demonstrate that no significant differences in scotopic and light-adapted F-ERG were found between toxin injected and control eye. In contrast, P-ERG response amplitudes evoked at 0.5 Hz or 4 Hz stimulation frequency showed a significant reduction in the toxin injected eye. Blocking α6-containing receptors at retinal level, also decreased VEPs amplitude recorded at different spatial frequencies in the visual cortex of contralateral injected eye. Moreover, using the fluorescently tagged toxin, we found a predominant distribution of labeling at the level of the ganglion cell layer. Our findings indicate that blocking α6-containing receptors leads to reduced visual function. Since both the cortical and inner retina output were affected by the toxin injection, whereas the photoreceptors output is preserved, we conclude that the reduced visual response resulted from altered function of α6-containing receptors specifically present in the ganglion cell layer.

Disclosures: **D. Barloscio:** None. **E. Cerri:** None. **L. Domenici:** None. **R. Longhi:** None. **M. Moretti:** None. **C. Gotti:** None. **N. Origlia:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.12/A81

Topic: B.02. Ligand-Gated Ion Channels

Support: GM57481

Title: Nicotinic activity of arecoline, the psychoactive element of betel nuts, suggests a basis for habitual use and anti-inflammatory activity

Authors: *R. L. PAPKE[1], N. A. HORENSTEIN[2], C. STOKES[1];
[1]Dept Pharmacol & Therapeut, [2]Chem., Univ. Florida, Gainesville, FL

Abstract: Habitual chewing of "betel nut" preparations constitutes the fourth most common human self-administration of a psychoactive substance after alcohol, caffeine, and nicotine. The primary active ingredient in these preparations is arecoline, which comes from the areca nut, the key component of all such preparations, along other components such as betel vine leaves or flowers, slaked lime, and about 50% of the time, tobacco. Betel nut effects are usually characterized as being like drinking strong coffee, but an objective observation of betel users might suggest other dominant actions; most striking is the production of copious amounts of saliva, of a bright red color, staining the lips and gums. Arecoline is known to be a relatively non-selective muscarinic partial agonist, accounting for many of the overt peripheral and central nervous system effects. This muscarinic activity, however, is not likely to account for the addictive properties of the drug. We have discovered previously unknown effects of arecoline on select nicotinic acetylcholine receptor (nAChR) subtypes, including the two classes of nAChR most related to the addictive properties of nicotine, receptors containing α4 and β2 subunits and those which also contain α6 and β3 subunits. Expression of α6 and β3 subunits is largely restricted to dopaminergic neurons associated with nicotine reward. Arecoline is a partial agonist with about 6-10% efficacy for the α4* and α6* receptors expressed in *Xenopus* oocytes. Additionally, arecoline is a silent agonist of α7 nAChR; while it does not activate α7 receptors when applied alone, it produces substantial activation when co-applied with the PAM PNU-120696. Methacholine and, to a lesser degree, Oxotremorine are also α7 silent agonists, while muscarine is not, providing insight into the structural requirements for α7 silent agonism. Some α7 silent agonists are effective inhibitors of inflammation, which might account for published reports of anti-inflammatory/immunosuppressive effects of arecoline. Recent epidemiology has indicated that long-term use of betel preparations is a major cause of oral cancer and other diseases of the mouth. Arecoline's activity on nAChR associated with addiction may account for the habitual use of areca nut preparations in spite of the well-documented risk to personal health. This common link between betel and tobacco suggests that partial agonist therapies with cytisine or the related compound varenicline may also be used to aid betel cessation attempts.

Disclosures: R.L. Papke: None. **N.A. Horenstein:** None. **C. Stokes:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.13/A82

Topic: B.02. Ligand-Gated Ion Channels

Support: The Research Funds of University of Helsinki (OS, a three-year grant)

Title: Chronic opioid treatment downregulates alpha4beta2 nicotinic acetylcholine receptors while upregulating alpha3* and alpha7 nicotinic acetylcholine receptors in cell lines

Authors: *R. TALKA, O. SALMINEN, R. K. TUOMINEN;
Univ. of Helsinki, Helsinki, Finland

Abstract: Although both opioids and nicotine have their own specific mechanisms of action, there is empirical and experimental evidence of *in vivo* and *in vitro* interactions between these drugs. Our previous *in vitro* studies in cell lines suggested that morphine acts as a partial agonist at α4β2 nicotinic acetylcholine receptors (nAChRs) and as a weak antagonist at α3* nAChRs, whereas methadone acts as an agonist at α7 nAChRs and a non-competitive antagonist at α4β2 and α3* nAChRs. Next we wanted to study the effect of the sub-chronic nicotine or opioid treatment on 3H-epibatidine binding in SH-EPI-hα4β2, SH-EPI-hα7 and SH-SY5Y (expressing α3* and α7 nAChRs) cell lines. The cell cultures were treated for 3 days either with 1 and 10 μM nicotine, methadone or morphine. On the day of assay, cultures were washed for 3×10 min with warm medium; then incubated for 3 h at 37°C before a final wash with warm PBS to remove all traces of drugs from the cultures. Homogenates were prepared and saturation binding assays were conducted as previously described in Talka et al. 2013. Nicotine (10 μM) upregulated 3H-epibatidine binding sites in all cell lines studied, confirming earlier well established paradigm. Both methadone and morphine upregulated the epibatidine binding sites in SH-EPI-hα7 and SH-SY5Y cell lines, the effect being more prominent at the SH-EPI-hα7 cell line with the dose of 10 μM. On the other hand, in SH-EPI-hα4β2 cell line both methadone and morphine downregulated the epibatidine binding sites, and the effect of sub-chronic morphine was especially pronounced in this cell line. These results add further strength to the notion that nicotine and opioids have indeed receptor-level interactions in *in vitro* settings.

Disclosures: R. Talka: None. **O. Salminen:** None. **R.K. Tuominen:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.14/A83

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH intramural research program

Title: α7 nicotinic acetylcholine receptor-mediated intracellular cAMP changes in hippocampal dentate gyrus neurons

Authors: *Q. CHENG[1], P. W. LAMB[2], J. L. YAKEL[2];
[1]Neurobio. Lab., [2]NIEHS, Durham, NC

Abstract: The activation of α7 nicotinic acetylcholine receptors (nAChRs) has been shown to improve hippocampal-dependent learning and memory. α7 nAChRs are widespread among different cell types in the hippocampus. However it is not known if α7 nAChRs mobilize differential signaling mechanisms within the same cell type or among distinct neuronal populations. Recently, we observed α7 nAChR-mediated cAMP rises using a FRET-based biosensor (TEpacVV) in cultured hippocampal neurons. Here, we are going to probe α7 nAChR's differential effects by employing CRE-dependent transgenic mouse lines. We created an AAV virus containing either an improved cAMP sensor (higher sensitivity and smaller size for better distribution) or GCaMP6s in a floxed configuration. We observed that the biosensor expression pattern was in fact limited to granule cell populations after infection of cultured hippocampal slices from these POMC-cre (dentate granule cells specific cre line) mice with the AAV virus. We found that application of the α7 nAChR-selective agonist choline (2 mM; in the presence of the α7 nAChR positive allosteric modulator PNU-120596 (5 μM)) induced a significant change in the YFP/CFP ratio in granule cell bodies, which indicated an increase in intracellular cAMP levels. GCaMP6 imaging revealed robust calcium rises induced by choline and PNU-120596 application in most granule cells. We will determine the heterogeneity of α7 nAChR-induced calcium responses in future analysis. To determine the differential effects of α7 nAChR activation among cell types, we will compare α7 nAChR agonist-induced calcium and cAMP changes between granule cells (POMC-cre) and GABAergic neurons (GAD-cre). We will correlate the changes in cAMP levels with the change of calcium and level of calcium-dependent adenylyl cyclases (ACs). Our findings may provide better understanding of the complex molecular mechanisms of the positive cognitive effects of α7 nAChR agonists in the brain, and strategies for the development of therapeutic treatments for cognitive impairments.

Disclosures: Q. Cheng: None. **P.W. Lamb:** None. **J.L. Yakel:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.15/A84

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS 1R15NS090368-01

Title: The beta2 subunit C loop of the nicotinic acetylcholine receptor directs allosteric modulator specificity

Authors: *M. M. LEVANDOSKI, A. R. MACK, C. A. SIBBALD;
Grinnell Col., Grinnell, IA

Abstract: Many compounds allosterically modulate neuronal nicotinic acetylcholine receptors (nAChRs), and novel modulators continue to be developed for use in pathologies involving nAChRs, such as nicotine addiction. We study nicotinic receptors heterologously expressed in *Xenopus* oocytes, primarily by electrophysiology. We showed that a pocket in the β(+)/α(–) interface of mammalian α3β2 nAChRs, homologous to the canonical ACh [α(+)/β(–)] site, is a novel binding site for the anthelmintics morantel and oxantel. We have identified several residues necessary for constituting this binding site and determining its specificity; the anthelmintics generally potentiate α3-containing receptors but inhibit α4-containing receptors. We hypothesize that discrete interactions between the C loop of the β2 subunit and residues on the α3(–) face direct selectivity between morantel and oxantel. Co-expressing mutant β2 subunits with a cysteine substitution in the C loop and a paired α3(–) cysteine mutant yields receptors with efficacy susceptible to modification by oxidation and reduction. We have studied a total of eight double-cysteine mutant pairs, combining two positions at the tip of the β2 C loop and four positions in the α3(–) face. Importantly, for a subset of these, the single α3(–) cysteine substitution (e.g. α3K55Cβ2) does not show modulator selectivity, but a double cysteine mutant (e.g. α3K55Cβ2S192C) does. We are extending this analysis to oxidation/reduction perturbations. We predict these experiments will show that the presence of modulator during oxidation treatment prevents disulfide trapping either through steric occlusion or movement of the cysteines to positions unfavorable for reaction, or promotes disulfide bond formation by minimization of the separation of the cysteines. These discrete contacts may differ for morantel and oxantel, thereby contributing to the modulator specificity differences.

Disclosures: M.M. Levandoski: None. **A.R. Mack:** None. **C.A. Sibbald:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.16/A85

Topic: B.02. Ligand-Gated Ion Channels

Title: Cognitive enhancement through augmentation of alpha7 nicotinic acetylcholine receptor function: *in vitro* and *in vivo* characterization of alpha7 agonist EVP-6124 and alpha7 positive allosteric modulator JNJ-39393406

Authors: *M. GRUPE[1], K. FREDERIKSEN[2], M. JESSEN[2], J. FULLERTON STØIER[3], A. PARACHIKOVA[4], C. BUNDGAARD[5], A. MITTOUX[6], J. BASTLUND[4];
[1]Synaptic Transmission *In vivo*, H. Lundbeck A/S, Valby, Denmark; [2]Mol. Pharmacol., [3]Neurodegeneration *In vivo*, [4]Synaptic Transmission *In vivo*, [5]Discovery DMPK, [6]Psychotic Disorders, H. Lundbeck A/S, Valby, Denmark

Abstract: The alpha7 nicotinic acetylcholine receptors (nAChRs) are homomeric ligand-gated ion channels expressed in brain regions of key importance for cognition, such as the prefrontal cortex and the hippocampus. Through modulation of neurotransmitter release these receptors are hypothesized to be essential for neurotransmission underlying higher cognitive functions, such as memory and attention (Lendvai *et al*., 2013). Additionally, altered expression and function of alpha7 nAChRs has been linked to various brain disorders such as schizophrenia (Freedman *et al*., 1995) and Alzheimer's Disease (Parri *et al*., 2011). These observations have formed the hypothesis that cognitive functioning can be improved by pharmacologically augmenting alpha7 nAChR function, thus making this receptor subtype a highly investigated drug target (Dineley *et al*., 2015). Employing *in vitro* and *in vivo* assays we have here investigated two alpha7 ligands of different pharmacological classes: EVP-6124 is an alpha7 agonist (Prickaerts *et al*., 2012), whereas JNJ-39393406 is an alpha7 positive allosteric modulator (PAM) (Winterer *et al*., 2013). Using two-electrode voltage clamping in *Xenopus* oocytes expressing human alpha7 nAChRs EVP-6124 was confirmed to be an agonist: at +10 nM EVP-6124 evokes a current response at the alpha7 nAChR and at 1 and 3 nM EVP-6124 has co-agonistic properties by potentiating ACh-evoked (30 μM) responses. JNJ-39393406 was found to be a potent and efficacious alpha7 PAM with EC_{50} = 6.3 μM at 30 μM ACh. The pro-cognitive potential of the compounds (0.1/1/10/100 mg/kg) were investigated in the mouse T-maze continuous alternation task (T-CAT) with acute PCP as a disrupter and the rat novel object recognition task (NOR) using a natural forgetting paradigm. At 0.1, 1 and 10 mg/kg EVP-6124 increased alternation scores, whereas only 10 mg/kg increased NOR performance. JNJ-39393406 improved T-CAT performance at only 0.1 and 100 mg/kg, and NOR performance at 1 and 100 mg/kg. This indicates that both compounds can enhance cognitive ability in rodents: EVP-6124 at defined dose ranges, whereas JNJ-39393406 displays a U-shaped dose-response curve. Finally, modulation of local field potentials by EVP-6124 and JNJ-39393406 in rat cortex in various auditory paradigms was investigated as measured by electroencephalogram electrodes. In summary, our findings demonstrate distinct pharmacological profiles of EVP-6124 and JNJ-39393406 on the molecular, synaptic, as well as behavioral level.

Disclosures: **M. Grupe:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **K. Frederiksen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **M. Jessen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **J. Fullerton Støier:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **A. Parachikova:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **C. Bundgaard:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **A. Mittoux:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **J. Bastlund:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.17/A86

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant NS59910

Title: Antidyskinetic effect of the novel α7 nicotinic receptor agonist ABT-126 in Parkinsonian monkeys

Authors: M. MCGREGOR[1], *D. ZHANG[1], T. BORDIA[1], X. A. PEREZ[1], M. W. DECKER[2], M. QUIK[1];
[1]SRI Intl., Menlo Park, CA; [2]AbbVie Inc., North Chicago, IL

Abstract: Recent evidence suggests that the nicotinic cholinergic system represents a target for antidyskinetic therapy in Parkinson's disease. Several studies show that the general nicotinic acetylcholine receptor agonist nicotine, as well as drugs acting at β2 or α7 nicotinic receptors, reduce levodopa-induced dyskinesias 60-70% in parkinsonian nonhuman primates, with no worsening of parkinsonism. Here we used parkinsonian monkeys to test the antidyskinetic effect of ABT-126, a novel α7 nicotinic receptor drug that has demonstrated an efficacy signal in a phase 2 study for Alzheimer's disease. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned monkeys were gavaged with levodopa (10 mg/kg)/carbidopa (2.5 mg/kg) twice daily. They were then given ABT-126 or vehicle orally 30 min before levodopa twice daily for 1-2 wk at each ABT-126 dose. A third group of monkeys was given nicotine as a positive control. ABT-126 (0.03, 0.10, 0.30 and 1.0 mg/kg) administration resulted in a dose-dependent decline in dyskinesias with ~60% decrease. A comparable reduction in dyskinesias was observed with both the morning and afternoon levodopa dose, with the latter associated with higher dyskinesia scores. There was no effect of ABT-126 on parkinsonism or cognition. No emesis was observed, a problem with other nicotinic receptor drugs. The effect of ABT-126 was relatively long-lasting, with a 6-week drug discontinuation period required for dyskinesias to return to the levels observed in vehicle-treated monkeys. These data suggest that the novel α7 nicotinic receptor agonist ABT-126 may be useful for the treatment of dyskinesias in Parkinson's disease.

Disclosures: **M. McGregor:** None. **D. Zhang:** None. **T. Bordia:** None. **X.A. Perez:** None. **M.W. Decker:** A. Employment/Salary (full or part-time):; AbbVie, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie, Inc. **M. Quik:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.18/A87

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS59910

Title: Striatal cholinergic interneurons regulate L-dopa-induced dyskinesias

Authors: *T. BORDIA, X. A. PEREZ, D. ZHANG, M. QUIK;
Ctr. for Hlth. Sci., SRI Intl., Menlo Park, CA

Abstract: L-dopa-induced dyskinesias (LIDs) are a serious complication of L-dopa-therapy for Parkinson's disease for which the neuronal circuitry and cellular mechanisms remain elusive. Recent work suggests a role for the cholinergic system as studies show that treatment with nicotinic cholinergic receptor (nAChR) drugs and ablation of cholinergic interneurons (ChIs) reduce LIDs in parkinsonian animals. Here we used optogenetics as an approach to examine the link between ChI activation and the expression of LIDs. For these studies, transgenic mice expressing cre-recombinase under the control of the choline acetyltransferase promoter (ChAT-cre mice) were lesioned by unilateral injection of 6-hydroxydopamine into the medial forebrain bundle. Two to four weeks later, AAV-DIO-ChR2-eYFP or DIO-eYFP was injected intrastriatally to elicit expression of ChR2-eYFP or eYFP in ChIs and optical fibers were implanted for *in vivo* stimulation. Mice were rendered dyskinetic by once daily injection of L-dopa. Three to four weeks were allowed for stable expression of the virus and L-dopa induced abnormal involuntary movements (AIMs). The mice were then subjected to various stimulation protocols (0.001 s to 20 s laser on, followed by 0.5 s laser off) while dyskinetic for a 2 h period. None of the stimulation regimens affected L-dopa-induced AIMs in mice expressing control virus. In mice expressing ChR2, shorter duration stimulation of 0.001 or 0.005 s enhanced L-dopa-induced AIMs compared to the unstimulated condition. By contrast, longer duration stimulation of 0.02 s resulted in a ~50% reduction in AIMs with comparable results with 1 s and 20 s stimulation. This decline in AIMs with light stimulation was similar to our previously observed decrease in L-dopa-induced AIMs with nicotine treatment. Thus, we next tested if the stimulation-induced changes in L-dopa-induced AIMs were nAChR-mediated. The general nAChR antagonist, mecamylamine blocked the decline in AIMs with longer (0.020 s) stimulation but not the increase observed with shorter (0.005 s) duration stimulation. In addition, parkinsonism on or off L-dopa was not affected by any of the stimulation paradigms suggesting that cholinergic transmission primarily regulates the expression of L-dopa-induced AIMs. In summary, shorter duration stimulation protocols that result in small bursts of acetylcholine increased L-dopa-induced AIMs, while longer duration stimulus protocols that lead to more prolonged acetylcholine release reduced AIMs. Overall, these results suggest that striatal ChIs play a critical role in LIDs and support the idea that decreases in LIDs occur via nAChR desensitization.

Disclosures: **T. Bordia:** None. **X.A. Perez:** None. **D. Zhang:** None. **M. Quik:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.19/A88

Topic: B.02. Ligand-Gated Ion Channels

Support: Pasteur Paris University International PhD programme

CNRS

ANR

Pasteur Innov

Stavros Niarchos Foundation

Title: Nicotinic receptors control prefrontal cortex activity

Authors: F. KOUKOULI[1], M. ROOY[3], B. GUTKIN[3], K. SAILOR[1], J. STITZEL[4], D. DIGREGORIO[1], *U. MASKOS[2];
[1]Inst. Pasteur, Paris cedex 15, France; [2]Inst. Pasteur, Paris Cedex 15, France; [3]ENS, Paris, France; [4]Univ. Colorado, Boulder, CO

Abstract: The prefrontal cortex (PFC) is integral for high order cognitive processes that can be modulated by cholinergic inputs via nicotinic acetylcholine receptors (nAChRs). Using *in vivo* two-photon imaging of awake mice, combined with network modeling, we show that nAChRs subtypes strongly, but differentially, regulate layer II/III pyramidal neuron activity. Both the interneuron-specific α7 and β2 subunits modulate pyramidal neuron activity by altering their inhibitory control. Efficient lentiviral vector-mediated re-expression of functional β2-subunit-containing nAChRs in PFC neurons of β2 knock-out (KO) mice rescued the neuronal activity to the levels of wild-type mice. We also demonstrate that the β2 subunit is specifically required for synchronized activity patterns. The local generation of sustained activity in neural networks of the cerebral cortex is associated with a striking balance of recurrent excitation and inhibition and, according to our data, this balance is disrupted in the β2 KO neural populations. Furthermore, mice expressing the human single nucleotide polymorphism (SNP) rs16969968 of the α5 subunit, which predisposes to nicotine addiction and schizophrenia, exhibit loss of pyramidal cell activity similar to α5 KOs. Our data suggest that interneuron specific expression of distinct nAChR types enables a diverse and bidirectional regulation of cortical activity and could provide a mechanistic basis for understanding the pathophysiology of genetically linked psychiatric disorders, as well as for identifying drug targets.

Disclosures: F. Koukouli: None. **M. Rooy:** None. **B. Gutkin:** None. **K. Sailor:** None. **J. Stitzel:** None. **D. DiGregorio:** None. **U. Maskos:** None.

Poster

292. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 292.20/A89

Topic: B.02. Ligand-Gated Ion Channels

Support: ANR-10-LABX-0087 IEC and ANR-10-IDEX-0001-02 PSL*

Grant/Other Support: : Government of the Russian Federation

Instiutu Pasteur

CNRS

INSERM

Title: Circuit level mechanisms enable the control of prefrontal cortex activity by nicotinic receptors

Authors: M. ROOY[1], F. KOUKOULI[2], D. DIGREGORIO[3], U. MASKOS[2], *B. S. GUTKIN[4,5]; [1]Group for Neural Theory, Lab. de Neurosciences Cognitives, INSERM Unité 969, Dept. d'É, Ecole Normale Superieure, Paris, France; [2]Integrative Neurobio. of Cholinergic Systems, CNRS UMR 3571, [3]Dynamic Neuronal Imaging, CNRS UMR 3571, Inst. Pasteur, Paris, France; [4]Group For Neural Theory, LNC INSERM U960, Ecole Normale Superieure, Paris, France; [5]Ctr. for Cognition and Decision Making,, Natl. Res. Univ. Higher Sch. of Econ., Moscow, Russian Federation

Abstract: Neurons in the prefrontal cortex (PFC) and its sub-areas receive significant cholinergic innervation, and a proportion expresses nicotinic acetylcholine receptors (nAChRs). By using *in vivo* two-photon imaging of awake mice, we showed that nAChR subtypes expressed only by interneurons in layer II/III, strongly modulate pyramidal neuron activity. In order to determine the specific circuit level mechanisms for such interneuron specific and nAChR type-specific bidirectional regulation of cortical activity we developed a minimal computational model of the local PFC circuitry. The model was built in order to implement the firing rate time evolution seen in the pyramidal and interneuronal populations of the wild type mice prefrontal cortex. Model parameters were fitted in order to yield the up and down state properties seen experimentally in the different neuronal types. We further incorporated nicotinic receptors models located on specific interneurons in the circuit. Three different subtypes of interneurons were explicitly modelled, in order to reproduce the effects of the different nicotinic subunits. In the model, the Parvalbumin interneuronal population expressed α7 nicotinic subunits, and interacted with the pyramidal population through substractive inhibition. The Somatostatin interneuronal population expressed α7 and β2 nicotinic subunits, and interacted with the pyramidal population through divisive inhibition. The VIP interneural population expressed α5 nicotinic subunits and targeted both Parvalbumin and Somatostatin interneuron populations through substractive inhibition. The model allowed us to account for the the firing rate changes and up-down states transitions seen experimentally in the knock out animals for the α7, α5 and

β2 nicotinic subunits. Analysis of the model allowed us to identify specific contributions of each receptor subtype to the control of the sustained activity dynamics. Our modeling pin-points specific points of entry for the pleotropic mechanisms by which nAChRs control the balance of excitation and inhibition in prefrontal cortical circuits. This approach allows us to delve into the role of nAChRs in the dynamic neural mechanisms underlying fronto-cortically dependent cognitive phenomena such as working memory, attention and cognitive control as well as in fronto-cortical pathologies.

Disclosures: **M. Rooy:** None. **F. Koukouli:** None. **D. DiGregorio:** None. **U. Maskos:** None. **B.S. Gutkin:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.01/A90

Topic: B.09. Network Interactions

Support: ONR N00014-13-1-0297

Title: Building a large-scale cortical network model incorporating laminar structure: frequency-specific feedforward and feedback interactions

Authors: ***J. F. MEJIAS**[1], J. D. MURRAY[1], H. KENNEDY[2], X.-J. WANG[1,3];
[1]Ctr. for Neural Science, New York Univ., New York, NY; [2]Stem-Cell and Brain Res. Institute, INSERM and Univ. de Lyon, Lyon, France; [3]NYU-ECNU Inst. of Brain and Cognitive Science, NYU Shanghai, Shanghai, China

Abstract: A major challenge in the development of large-scale cortical network models is to implement area-to-area interactions allowing elucidation of dynamical operations in a biologically constrained manner. These interactions are anatomically organized in a laminar specific manner: feedforward projections preferentially stem from superficial layers, whereas feedback projections originate chiefly from deep layers. Feedforward interactions transmit sensory information to higher brain areas, while feedback interactions may mediate a prediction/expectation signal or top-down attentional modulation of early sensory areas. Quantitative data on laminar-dependent inter-areal connectivity of the macaque cortex have become available only recently. We have incorporated these data in a large-scale dynamical model of the primate cortex endowed with weighted and directed connectivity. Each cortical area is modeled with a superficial and a deep layer. Based on recent physiological evidence, we modeled excitatory and inhibitory neural populations in each layer, with local properties that generate noisy gamma oscillations in the superficial layer and alpha oscillations in the deep layer. Furthermore, the interactions between the two layers are guided by anatomical and

physiological data, with specific superficial-to-deep and deep-to-superficial projections that allow to explain experimental observations of phase-amplitude coupling. We calibrated the model by simulating physiological observations that feedforward interactions are associated with oscillations in the gamma band (40-80Hz), while feedback interactions relate to lower frequencies, in the alpha or low beta frequency range (8-20 Hz). Using Granger causality to establish the directionality of information flow, the model reproduces the observed functional hierarchical order of visual areas (Bastos et al. Neuron 2015). The model identifies several properties of feedback projections as a key factor to explain these hierarchical dynamics, in particular the specific pattern of feedback projections to a target area. We further discuss the usefulness of functional hierarchies to reconstruct structural properties of cortical connectivity. Our results represent a step forward in the advance of a quantitative model of the primate large-scale cortical system.

Disclosures: **J.F. Mejias:** None. **J.D. Murray:** None. **H. Kennedy:** None. **X. Wang:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.02/A91

Topic: B.09. Network Interactions

Title: Diversity of sharp wave-ripples in the CA1 of the macaque hippocampus and their brain wide signatures

Authors: *J. F. RAMIREZ-VILLEGAS[1,2], N. K. LOGOTHETIS[1,3], M. BESSERVE[1,4]; [1]Physiol. of Cognitive Processes, Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany; [2]Grad. Sch. of Neural & Behavioral Sci., Eberhard-Karls Univ. Tuebingen, Tuebingen, Germany; [3]Ctr. for Imaging Sciences, Biomed. Imaging Inst., The Univ. of Manchester, Manchester, United Kingdom; [4]Dept. of Empirical Inference, Max Planck Inst. For Intelligent Systems, Tuebingen, Germany

Abstract: Sharp wave-ripple complexes are thought to play a major role in memory reactivation, transfer and consolidation. However, the large-scale cooperative mechanisms associated to these episodes and their relationship to the observed SPW-R electrical signature remains largely unknown. A better understanding of the underlying mechanisms of these interactions requires a finer characterization of the SPW-R phenomenon and its associated brain-wide signatures. To address this question, we hypothesize that SPW-R dynamics vary, reflecting distinct interactions with neocortical and subcortical systems depending on the state of the animal. Specifically, the wide-range network reconfiguration required by this process may bring different electrical signatures of SPW-Rs, thus reflecting different memory-related functional roles. Using concurrent hippocampal local field potentials (LFP) recordings and functional Magnetic

Resonance Imaging (fMRI) in anesthetized macaques, we study local changes in neuronal activity during SPW-R episodes and their brain-wide correlates. After detecting SPW-R episodes based on power increases in the ripple frequency band (80-180 Hz), analysis of peri-event SPW-R complexes reveals four well-differentiated SPW-R subtypes in the CA1 LFP. Event-triggered fMRI maps show that SPW-R subtypes relate to differentiated multi-structure activity (MSA). We found that ripples aligned to the positive peak of their SPWs were associated with significantly higher BOLD up-regulations within the hippocampal formation, and in cortical associative areas (namely, anterior cingulate cortex, retrosplenial area, prefrontal, temporal and parietal cortices), as compared to ripples occurring at the trough of their SPW ($p<0.01$ Wilcoxon rank-sum test, FDR-corrected with $q<0.05$). Conversely, detailed analysis of all subcortical domains revealed differentiated BOLD activations in locus coeruleus (LC) and dorsal raphe nucleus ($p<0.002$, pairwise Wilcoxon rank-sum test, FDR-corrected with $q<0.05$), suggesting that emergence of different SPW-R signatures may be influenced by state-dependent neuromodulatory inputs of differentiated nature into the hippocampal formation. Altogether, our results suggest that the variability of CA1 SPW-R episodes reflect different levels of activation over cortical and subcortical domains. We hypothesize that these distinct patterns of SPW-R complexes reflect brain-wide cooperative events, possibly involved in different memory-related functions.

Disclosures: **J.F. Ramirez-Villegas:** None. **N.K. Logothetis:** None. **M. Besserve:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.03/A92

Topic: B.09. Network Interactions

Support: NEI Grant R01EY011787

HFSP Long Term Fellowship

Title: VIP and SOM interneurons compete to cooperate

Authors: ***M. M. KARNANI**, J. C. JACKSON, I. AYZENSHTAT, R. YUSTE;
Biol. Sci., Columbia University, Dept. of Biol. Sci., New York, NY

Abstract: The canonical cortical interneuron circuit involving cardinal interneuron populations is becoming clear. Two major neocortical interneuron populations defined by expression of somatostatin or vasoactive intestinal peptide powerfully inhibit each other while inhibitory connections within these populations are highly infrequent. We used quadruple patch clamp recordings in triple-transgenic mouse brain slices to obtain synaptic parameters and network statistics of these and other previously undocumented synaptic connections among genetically

defined interneuron populations and pyramidal cells. Our results show multiple specializations for well-organized competition between interneuron populations as well as cooperativity within populations. *In vivo* calcium imaging data indicates that these forces are at play in the awake brain as well as in slices, resulting in a high tendency for coactivity within interneuron populations. Supported by the NEI (R01EY011787) and the HFSP.

Disclosures: **M.M. Karnani:** None. **J.C. Jackson:** None. **I. Ayzenshtat:** None. **R. Yuste:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.04/A93

Topic: B.09. Network Interactions

Support: NIH Grant K25-DA021200

NIH Grant R21-DA032228

NIH Grant R01-DA029718

NIH Grant R01-NS084817

China 863 Grant 2012AA022402

China 973 Grant 2013CB733801

Title: Neuronal connectivity at resting-state decreased in cortex induced by cocaine

Authors: *G. XIAOCHUN[1,2], J. CHOI[1], N. VOLKOW[3], Y. PAN[1], C. DU[1];
[1]Dept. of Biomed. Engineering, State Univer, Stonybrook, NY; [2]Key Lab. of Developmental Genes and Human Diseases, Dept. of Anat. and Neuroscience, Med. School, Southeast University, Nanjing, 210009, PR China, Nanjing, China; [3]Natl. Inst. on Drug Abuse, NIH, Bethesda, MD

Abstract: Neuroplastic changes in the cortex are implicated in the behavioral disruption observed with substance use disorders. Imaging of functional brain connectivity during the resting-state is increasingly utilized to investigate how drugs affect systems-level brain networks. Although resting-state MRI has provided significant new knowledge on the changes in functional brain connectivity induced by cocaine, it is still a challenge to directly image temporal changes in neuronal activity, which is important when investigating the effects cocaine, which not only affects neuronal activity but also has direct vasoactive effects. Here, we present our optical imaging technique in combination with the genetic calcium labeling (GCamp6f) to study the

neuronal Ca activities in sensorimotor cortex at resting-state; we assessed connectivity across different cortical regions and layers and evaluated cocaine's effects in the connectivity patterns. Two groups of animal were used: Group 1 (n=5), we virus-delivered GCamp6f into the forepaw (FP), hindpaw (HP) and barrel (BC) cortex in Layer II-III (~ 250μm depth), whereas in Group 2 (n=6), the GCamp6f was injected in Layer IV-V of the same cortex (~ 500μm depth). Four weeks late, the animals were imaged with optical/fluorescence imaging (OFI) to simultaneously record the neuronal Ca2+ changes along with the spontaneous hemodynamic fluctuations in the cortex from baseline to cocaine. We also compared the effects in different cortical layers. Our results showed that, the autocorrelations in local FP, HP or BC areas at resting-state were >80% in both upper and deeper cortical layers, thus indicating that local neuronal activities were highly correlated. The cross-correlations between FP, HP and BC cortices were ~70% in the baseline and decreased after cocaine in upper layers (Layer II-III). Specifically, the cross-correlations of BC vs FP, BC vs HP were decreased ~53% (i.e., from 0.77±0.05 to 0.36±0.1, p=0.006) and ~48% (i.e., from 0.76±0.09 to 0.4±0.1, p=0.008), respectively, and those between FP and HP were decreased ~21% (i.e., from: 0.88±0.03 to 0.69±0.07, P=0.04) after cocaine in the upper cortical layers. Interestingly, the cross-correlations between different areas in the deeper cortical layers were not changed after cocaine. The reductions in cross-correlation indicates that neuronal functional connectivity was decreased by cocaine, which might contribute to impaired processing of cortical information during cocaine intoxication.

Disclosures: G. Xiaochun: None. **J. Choi:** None. **N. Volkow:** None. **Y. Pan:** None. **C. Du:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.05/A94

Topic: B.09. Network Interactions

Support: BBSRC Grant BB/J015369/1

Title: Spatio-temporal dynamics of network activity coupled to the action of the neuromodulator adenosine

Authors: *M. J. RICHARDSON, F. FERMANI, A. NEWTON, M. THOMAS, M. WALL; Univ. of Warwick, Coventry, United Kingdom

Abstract: We combine experimentally verified models of neocortical-neuron voltage dynamics and network connectivity to derive a framework that describes activity at a tissue scale. The resulting description represents a neuronal field theory in which emergent properties at the coarse-grained level can be causally linked to the physiology of cellular and sub-cellular

components. The description is solvable and also straightforward to simulate, and can be elaborated to include further biophysical details such as multiple neuronal populations to capture the structure of the component microcircuits, synaptic dynamics and filtering as well as distance-dependent delays in signal propagation. We demonstrate the utility of the approach by modelling recent experimental results on the negative feedback coupling between the activity-dependent release of the neuromodulator adenosine, its diffusion in the extracellular space and its subsequent suppression of neocortical activity.

Disclosures: **M.J. Richardson:** None. **F. Fermani:** None. **A. Newton:** None. **M. Thomas:** None. **M. Wall:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.06/A95

Topic: B.09. Network Interactions

Support: Helmholtz Portfolio Theme Supercomputing and Modeling for the Human Brain (SMHB)

EU grant 604102 (Human Brain Project, HBP)

ANR-GRASP

Neuro_IC2010

CNRS-PEPS

Riken-CNRS Research Agreement

Title: Relating spatial patterns of beta oscillations to their power in macaque motor cortex: a case study in using the "Elephant" data analysis framework in a reproducible analysis workflow

Authors: ***M. DENKER**[1], L. ZEHL[1], B. KILAVIK[2], M. DIESMANN[1], T. BROCHIER[2], A. RIEHLE[2,1,3], S. GRÜN[1,4,3];
[1]Inst. of Neurosci. and Med. (INM-6) and Inst. for Adv. Simulation (IAS-6), Jülich Res. Ctr. and JARA, Jülich, Germany; [2]Inst. de Neurosciences de la Timone (INT), CNRS, Aix-Marseille Univ., Marseille, France; [3]RIKEN Brain Sci. Inst., Wako-Shi, Japan; [4]Theoretical Systems Neurobio., RWTH Aachen Univ., Aachen, Germany

Abstract: The unprecedented degree of complexity in electrophysiological experiments has reached a level where well-structured data analysis workflows have become a necessity. Here we introduce a case study that links emerging software tools to form a reproducible analysis

workflow. As a key component of such workflows, we introduce the Electrophysiology Analysis Toolkit ("Elephant", http://neuralensemble.org/elephant/) as a recent community-centered initiative to develop an analysis framework for multi-scale activity data based on common data representations provided by the Neo library [Garcia et al. (2014) Front. Neuroinform 8:10]. During states of increased arousal, motor preparation, and postural maintenance, the local field potential (LFP) in primary motor (M1) and premotor (PM) cortex typically exhibits oscillations in the beta (12-40 Hz) range [Kilavik et al. (2012) Cereb Cortex 22:2148]. Beta oscillations recorded on separate electrodes are often highly correlated, but exhibit a non-zero phase shift. These shifts were shown to organize spatially in the form of planar wave propagation along preferred directions across the cortical surface during an instructed-delay reaching task [Rubino et al. (2006) Nat Neurosci 9:1549]. In this case study we demonstrate that in monkey motor cortex a variety of additional spatial patterns of LFP beta activity may be distinguished outside epochs that exhibit a clear planar wave. We recorded massively parallel neuronal activity using a 10-by-10 Utah electrode array (Blackrock Microsystems), which was chronically implanted in M1 and dorsal PM. The monkey was trained in a delayed reach-to-grasp task [Riehle et al. (2013) Front Neural Circuits 7:48]. Based on the instantaneous phase and phase gradients of the beta-filtered LFP, we introduce and combine measures to identify different spatial activity patterns: (i) planar waves, (ii) quasi-stationary states (all electrodes appear synchronized at near-zero lag), (iii) spatially unstructured states, and (iv) more complex patterns, including circular and radial propagation. We assess the statistical properties of the patterns, including their duration and average direction. In particular, we relate the observed patterns to beta-spindles identified by large instantaneous amplitudes. We find that the wave pattern correlates with the beta power, where the peak of spindles typically coincides with a quasi-stationary state. In combination with previous results [Denker et al. (2011) Cereb Cortex 21:2681], this raises the hypothesis that beta power is indicative of spatio-temporal organization of spike synchronization.

Disclosures: **M. Denker:** None. **L. Zehl:** None. **B. Kilavik:** None. **M. Diesmann:** None. **T. Brochier:** None. **A. Riehle:** None. **S. Grün:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.07/A96

Topic: B.09. Network Interactions

Support: NIAAA Grant R01AA022821

RFBR Grant 14-02-00916-a

Title: Contribution of synchronized GABAergic neurons to dopaminergic neuron firing and bursting

Authors: *E. MOROZOVA[1,3], D. ZAKHAROV[5], M. MYROSHNYCHENKO[2], M. DI VOLO[3], B. GUTKIN[6,7], C. LAPISH[4], A. KUZNETSOV[3];
[1]Physics, Indiana Univ., Indianapolis, IN; [2]Program in Neurosci., Indiana Univ., Bloomington, IN; [3]Dept. of Mathematical sciences, [4]Addiction Neurosci. Program, Indiana University-Purdue Univ., Indianapolis, IN; [5]Inst. of Applied Physics, Nizhny Novgorod, Russian Federation; [6]Group of Neural Theory, École normale supérieure, Paris, France; [7]Theoretical Neurosci. Group, Ctr. for Cognition and Decision Making, Natl. Res. Univ. Higher Sch. of Econ., Moscow, Russian Federation

Abstract: Dopaminergic (DA) neurons *in vivo* are constantly bombarded by various excitatory and inhibitory inputs. A substantial proportion of the inhibitory drive to DA neurons in ventral tegmental area (VTA) comes from local GABA neurons, which modulates DA neuron firing and thus the amount of released DA throughout the brain. We investigated how synchronous activity of local VTA GABA neurons influence DA neuron firing patterns. We developed a local circuit model of the VTA consisting of one DA neuron innervated by a population of GABA neurons. The model was calibrated to reproduce dynamic clamp experimental results under application of tonic NMDA and GABA receptor currents. Particularly, burst firing in the model could be reproduced by application of NMDA or through disinhibition from GABA. Similar to the experimental results, background firing was inhibited by tonic GABA inputs to the DA neuron; however, phasic firing evoked by NMDA was preserved. In addition, well-established features of DA neurons modeled before, such as high frequency response to NMDA activation but relatively low firing rates when driven with applied current or AMPA receptor activation, were also reproduced in the model. To better approximate *in vivo* conditions, we explored how networks of GABA neurons modulate DA neuron firing. Modeling revealed that GABA neurons can differently influence the firing pattern of the DA neuron depending on the level of synchronization in across GABA networks. Asynchronous activity of GABA neurons in the population provides constant level of inhibition to DA neuron and acts very similarly to the tonic inhibition as described above. Under the combined influence of desynchronized glutamatergic and GABA populations, the DA neuron fires with frequencies close to those observed during background firing (in the absence of synaptic inputs) but less regularly. In contrast to asynchronous activity, coordinated activity of synchronized GABA neurons can evoke additional spikes in the DA neuron. By hyperpolarizing the cell membrane, GABA current decreases intracellular Ca concentration, because activation threshold for Ca current is -50 mV. With the drop in Ca level the calcium-dependent potassium current decreases. This leads to faster depolarization. In contrast to tonic inhibition, when the GABA population is synchronized inhibition becomes pulsatile providing the DA neuron an opportunity to fire between pulses. Our simulations suggest that synchrony amongst GABA neurons seems to be a critical intermediary that regulates DA neuron activity and, hence, DA release throughout the brain.

Disclosures: E. Morozova: None. **D. Zakharov:** None. **M. Myroshnychenko:** None. **M. di Volo:** None. **B. Gutkin:** None. **C. Lapish:** None. **A. Kuznetsov:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.08/A97

Topic: B.09. Network Interactions

Support: NIAAA grant 1R01AA022821

Title: Simulating the effects of ethanol on Ventral Tegmental Area local circuit dynamics and Dopamine neuron firing

Authors: *M. DI VOLO[1,2], E. MOROZOVA[1], M. MYROSHNYCHENKO[3], C. LAPISH[4], A. KUZNETSOV[5], B. GUTKIN[2,6];
[1]Dept. of Physics, IUPUI, Indianapolis, IN; [2]Group of Neural Theory, ENS, Paris, France; [3]Program in Neuroscience, Indiana Univ., Indianapolis, IN; [4]Addiction Neurosci. Program, IUPUI, Indianapolis, IN; [5]Dept. of Mathematical sciences, IUPUI., Indianapolis, IN; [6]Theoretical Neurosci. Group, Ctr. for Cognition and Decision Making, Natl. Res. Univ. Higher Sch. of Econ., Moscow, Russian Federation

Abstract: Experimental data show that alcohol consumption produces an increase of dopamine (DA) release from dopaminergic neurons of the Ventral Tegmental Area (VTA). This brain region, and the computational processes it performs, are crucial for processing information about rewarding stimuli and reinforcement learning, and hence plays a central role in addiction. Consistent with this view, rewarding events and salient stimuli each increase the firing rate of DA neurons, which is argued to play a central role in encoding information about expected rewards and their motivation salience. The primary goal of the current study is to describe and explore how acute ethanol exposure alters the biophysical properties of VTA DA neurons through a computational model of the VTA circuit. A simplified biophysical model of the DA neuron was first implemented to explore the effects of ethanol on firing in the absence of glutamatergic and GABAergic inputs. Towards this goal, changes in DA neuron excitability were quantified by measuring the maximal frequencies evoked by stimuli of varying sizes. Specifically, we show that potentiation of hyperpolarization activated, cyclic nucleotide gated (HCN) channels and activation of GIRK channels mostly cancel each other, and thus have opposing effects on the firing rate of the DA neuron. Furthermore, the HCN current in isolation (when GIRK channel is blocked) mostly affects low frequency firing, but not when firing rate is very high. Thus, these channels are not sufficient to increase the excitability of the DA neuron. Additional simulations identified a subthreshold sodium current as a possible target capable of increasing excitability of the DA neuron. We then investigated effects of ethanol when glutamatergic and GABAergic inputs are included in a local circuit model composed of the DA neuron and GABA population. As suggested by experimental data, we found that acute ethanol effects DA neuron firing through an increase in the AMPA/NMDA ratio, potentiation of GABA synapses on the DA neuron, and a decrease in GABA neuron firing rate. We describe how acute ethanol produces an increase in the number of spikes in bursts by its effect on GABAergic and

glutamatergic inputs received by DA neuron. Moreover, this effect depends on the burstiness of glutamatergic inputs on DA neuron firing when ethanol is applied. We discuss the optimal combination of the intrinsic and network mechanisms that contribute to the excitation of the DA neuron with the future goal of testing these hypotheses directly in experiments. This project is funded by NIAAA grant 1R01AA022821

Disclosures: **M. Di Volo:** None. **E. Morozova:** None. **M. Myroshnychenko:** None. **C. Lapish:** None. **A. Kuznetsov:** None. **B. Gutkin:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.09/A98

Topic: B.09. Network Interactions

Support: NIH Grant R01EB008085

NIH Grant R01EB009233

NIH Grant K25NS083754

NIH Grant T32EB014855

Title: Spontaneous calcium transients precede hemodynamic activity and produce homotopic functional connectivity maps

Authors: *P. WRIGHT, A. BAUER, G. BAXTER, J. CULVER;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Hemodynamic-based markers of cortical activity (e.g. fMRI, optical intrinsic signal (OIS) imaging) are an indirect report of brain function driven by electrical and metabolic activity through neurovascular coupling. Here we extend functional connectivity (FC) analysis, a method for mapping functional relationships using spontaneous brain activity, from hemodynamic to Ca2+-dynamic imaging. Transgenic mice (n=5) expressing a fluorescent calcium indicator (GCAMP3) driven by the Emx1 promoter in cortical glutamatergic neurons and glia were anesthetized using ketaminze/xylazine and imaged transcranially. Sequential LED illumination (λ=470, 530, 590, 625nm) enabled concurrent imaging of both GCAMP3 fluorescence emission (corrected for hemoglobin absorption) and hemodynamics. Somatosensory responses were evoked using a 0.5mA electrical hindpaw block paradigm. FC patterns were generated for low (0.009-0.08Hz) and high (0.2-2Hz) frequency bands. Following paw stimulation, GCAMP3 provided a response time course sensitive to individual high frequency (2Hz) pulse presentations and preceded the stereotypical hemodynamic response function by approximately 1.5 seconds (Fig. 1A&B). Within the high frequency band, pixelwise cross-correlation analysis of

spontaneous data revealed that GCAMP3 again preceded HbO2 by approximately 1.5 seconds across the brain (Fig 1C&D). Furthermore, homotopic FC maps remained intact in higher frequencies relative to HbO2 maps but had similar topography (Fig. 1E). In summary, functional neuroimaging of Ca2+ dynamics in mice provides evidence that spatiotemporal coherence in cortical activity is not exclusive to hemodynamics. This fast Ca2+ signal is more directly coupled to activity at the neuronal level and likely has causal information predictive of the downstream hemodynamic response. Concurrent Ca2+ and hemodynamic-based imaging will enable the dissociation of changes in ionic networks, hemodynamic networks, and neurovascular coupling and provide a framework for subsequent studies of neurological disease, such as stroke.

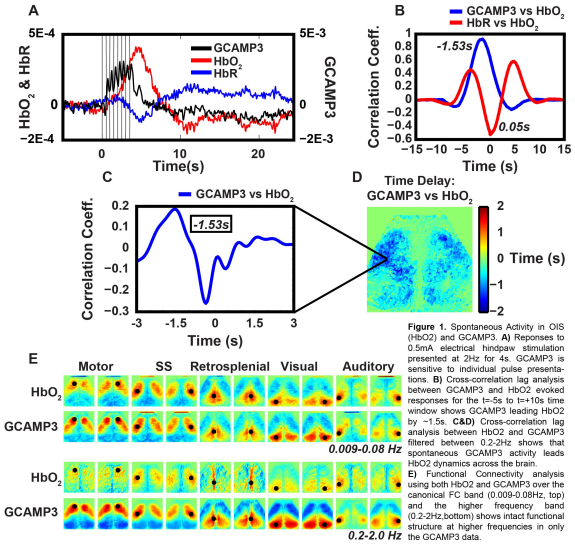


Figure 1. Spontaneous Activity in OIS (HbO2) and GCAMP3. **A)** Reponses to 0.5mA electrical hindpaw stimulation presented at 2Hz for 4s. GCAMP3 is sensitive to individual pulse presentations. **B)** Cross-correlation lag analysis between GCAMP3 and HbO2 evoked responses for the t=-5s to t=+10s time window shows GCAMP3 leading HbO2 by ~1.5s. **C&D)** Cross-correlation lag analysis between HbO2 and GCAMP3 filtered between 0.2-2Hz shows that spontaneous GCAMP3 activity leads HbO2 dynamics across the brain. **E)** Functional Connectivity analysis using both HbO2 and GCAMP3 over the canonical FC band (0.009-0.08Hz, top) and the higher frequency band (0.2-2Hz,bottom) shows intact functional structure at higher frequencies in only the GCAMP3 data.

Disclosures: **P. Wright:** None. **A. Bauer:** None. **G. Baxter:** None. **J. Culver:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.10/A99

Topic: B.09. Network Interactions

Title: Modulation of hippocampal gamma oscillation activity by histone acetylation and nuclear receptor family 4a in Alzheimer's disease model mice

Authors: K. TAKASU, K. NIIDOME, M. HASEGAWA, G. SAKAGUCHI, *K. OGAWA; SHIONOGI & CO., LTD., Toyonaka-Shi, Osaka, Japan

Abstract: Hippocampal gamma oscillation is reported to be associated with cognitive functions, and accumulating evidence suggest that deficit of gamma oscillation is related to cognitive

impairment in Alzheimer's disease (AD). Recent studies suggest that post-translational modification of histone protein via acetylation is a fundamental molecular mechanism for regulation of synaptic plasticity and memory formation. However, little is known about roles for histone acetylation in the hippocampal gamma oscillation. In this study, we investigated whether histone acetylation and its downstream signaling pathway, nuclear receptor family 4a (NR4a) regulate kainate-induced gamma oscillation by using acute hippocampal slices of AD model mice (PS/APP transgenic mice). We found that kainate-induced gamma oscillation in the hippocampal slices of PS/APP mice was decreased compared with that in control mice (C57BL/6J mice or PS mice), and application of donepezil, a clinically used drug for AD treatment, recovered the gamma oscillation. Decrease of gamma oscillation was also observed in the hippocampal slices of aged PS mice (14 months) and that of C57BL/6J mice (3 months) treated *in vitro* with amyloid beta$_{1\text{-}42}$. Then, we investigated the effects of histone deacetylase (HDAC) inhibitors (suberoylanilide hydroxamic acid (SAHA) and MS-275) and a NR4a activator (cytosporone B) on the gamma oscillation in PS/APP mice. We found that both SAHA and MS-275 elevated the gamma oscillation level, and the effect of SAHA was accompanied with the elevation of histone H3 and H4 acetylation. The effects of SAHA on the gamma oscillation and histone acetylation were abolished by co-application of histone acetyltransferase (HAT) inhibitor C646, showing that histone acetylation is a key mechanism of the observed SAHA effects. We further demonstrated that NR4a activation by cytosporone B improved the gamma oscillation in PS/APP mice. In C57BL/6J mice, SAHA increased gamma oscillation, while C646 alone abolished gamma oscillation. Our results indicate that histone acetylation modulates kainate-induced gamma oscillation in the hippocampal slices of both normal and AD model mice. As possible pathological mechanism of AD, histone acetylation may be suppressed by the elevated HDAC activity in the hippocampus. Reversal of gamma oscillation deficits by HDAC inhibition and/or NR4a activation appears to be a potential therapeutic approach to improve cognitive function in AD.

Disclosures: **K. Takasu:** None. **K. Niidome:** None. **M. Hasegawa:** None. **G. Sakaguchi:** None. **K. Ogawa:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.11/A100

Topic: B.09. Network Interactions

Support: Fondation pour la Recherche Médicale (FRM) postdoctoral fellowship to M.V

Fyssen foundation postdoctoral fellowship to JS

EU FP6 & ANR eraNET NEURON Beyondvis

IHU-A-ICM-Translational grant

"Naturalia et Biologia" Foundation

Title: Entrainment of local oscillatory activity in the human brain: Evidence from Intracranial multi-electrode stimulation recordings

Authors: J. AMENGUAL[1], M. VERNET[1], C. ADAM[2], *A. VALERO CABRE[3,1,4];
[1]CNRS UMR 7225, Inst. du Cerveau et de la Moelle, Cerebral Dynamics, Plasticity and Rehabil. Group, Frontlab, Paris, France; [2]Epilepsy Unit, Dept. of Neurology, Pitié-Salpêtrière Hosp., Pitié-Salpêtrière Hospital-APHP, Paris, France; [3]Dept. Anat. and Neurobio., Lab. Cerebral Dynamics, Boston Univ. Sch. of Med., Boston, MA; [4]Cognitive Neurosci. and Information Technol. Res. Program,, Open Univ. of Catalonia (UOC), Barcelona, Spain

Abstract: In the healthy brain, local and interregional oscillatory activity at specific frequency bands codes for the engagement of different cognitive operations and behaviors. In turn, specific neurological syndromes have been associated to particular alterations of oscillatory mechanisms. More recently, rhythmic Transcranial Magnetic Stimulation coupled to scalp electroencephalography (EEG) has provided evidence of local oscillation entrainment at a frequency band dictated by the stimulation input. These findings have spurred interest for the manipulation of local synchrony, either in search of causal associations between oscillations and brain functions or for the development of novel therapeutic approaches in the field of neuromodulation. However, direct evidence that overcomes the limitations of non-invasive stimulation and recording procedures remains paramount. We here analyzed intracranial EEG (iEEG) signals from multielectrodes implanted in the prefrontal cortex of fully awake epileptic patients (n=3) undergoing focal intracortical stimulation, as part of a clinical procedure aimed to identify the localization of epileptogenic loci. Responses to 5 seconds bursts of biphasic 50 Hz squared electrical pulses delivered at increasing intensities (0.5 to 5 mA) by pairs of adjacent contacts of the same multi-electrode were recorded. Changes in iEEG signals in absence of signs of epileptiform discharges, recorded prior, during and shortly after low intensity stimulation (up to 2 mA) were considered in our study. Our analyses provide direct evidence of 50 Hz oscillatory entrainment along the stimulation burst, characterized by power increases around the input frequency (45-55 Hz) and phase locking to the electrical stimulation signal. The magnitude of this gamma band entrainment depended on stimulation intensity and the distance between the contact pairs involved in the stimulation and the recording of iEEG data. Highlighting the physiological relevance of this phenomenon, the highest entrainment was found in areas with the highest level of synchronization with the stimulated site in absence of stimulation. Artificially modeled iEEG time series and noise served to rule out the confounding of electrical artifacts. Our results provide evidence that direct stimulation of brain tissues entrains physiological oscillatory activity at the input frequency. This outcome opens new avenues to explore the causal role of brain synchrony in specific cognitive processes. Furthermore, it will allow the further development of novel stimulation therapeutic approaches for neurological conditions subtended by dysfunctional oscillatory activity.

Disclosures: **J. Amengual:** None. **M. Vernet:** None. **C. Adam:** None. **A. Valero Cabre:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.12/A101

Topic: B.09. Network Interactions

Support: NIH MH060605 (FN)

NSF DMS1313861 (HGR)

Title: The effect of resonance frequency on network oscillations through electrical gap junction coupling

Authors: ***X. LI**[1], Y. CHEN[1], H. G. ROTSTEIN[2], F. NADIM[1,2];
[1]Dept Biol. Sci., Rutgers/Njit, Newark, NJ; [2]Dept Mathematical Sci., New Jersey Inst. of Technol., Newark, NJ

Abstract: Neurons often produce a maximal subthreshold voltage response to oscillatory current inputs at a non-zero input frequency (f_{res}), a property known as membrane potential resonance. Typically, resonance is measured by using the impedance function Z(f) of a neuron over a range of frequencies (f). Recent studies have suggested that f_{res} of constituent neurons is a strong indicator of the network frequency. Nevertheless, how the resonance property influence the network frequency remains unclear. We focus on networks of neurons that are electrically coupled through gap junctions and propose the hypothesis that, in such a network, parameters that shift the resonance frequency also shift the network frequency in the same direction. Additionally, we propose that an increase in the resonance power ($Q=Z(f_{res})/Z(0)$) of participating resonator neurons increases this dependence. We test our hypothesis in an electrically coupled network consisting of a pacemaker neuron (having intrinsic oscillations) and a resonator that does not necessarily have intrinsic oscillations. In a previous computational study of a two-cell model of such a network, we showed that it is the impedance profile of the resonator, not the specific neuron model parameters, that influences the network frequency (Chen et al, SfN Abst 538.24, 2014). We test this hypothesis using dynamic clamp technique in a biological network consisting of pacemaker neurons electrically coupled to a resonator neuron. In the oscillatory pyloric network of the crab *C. borealis*, the network frequency has been found to be correlated with the resonance frequency of its pacemaker group neurons (Tohidi & Nadim, *J Neurosci*, 2009). Additionally, although the non-pacemaker follower neurons do not produce oscillations, they also exhibit resonance but with a different f_{res} than that of the pacemaker neurons (Tseng et al, *J Neurosci*, 2014). To examine our hypothesis, we couple the anterior

burster (AB) and the pyloric dilator (PD) pacemaker group neurons in the pyloric network to a biophysical-inspired model resonator via gap junction using dynamic clamp. In the model resonator, the attributes f_{res} and Q can be varied independently by changing the parameters of the resonator neuron. We show that by shifting the f_{res} of the resonator without changing the shape of the impedance profile, the network frequency shifts in the same direction and this effect is enhanced by increasing the resonance power Q. Our results provide experimental support that resonance frequency and power can strongly influence the network oscillation frequency and therefore modulators may directly target these attributes in order to influence network activity.

Disclosures: **X. Li:** None. **Y. Chen:** None. **H.G. Rotstein:** None. **F. Nadim:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.13/A102

Topic: B.09. Network Interactions

Title: Modeling the effects of inhibitory and excitatory synchrony on seizure generation in a CA1 circuit

Authors: *J. R. CRESSMAN[1], D. B. DORMAN[2];
[2]Mol. Neurosci., [1]George Mason Univ., Fairfax, VA

Abstract: The CA1 region of the hippocampus has been implicated in temporal lobe epilepsy. Factors underlying seizure generation and propagation are complex and may depend on the interaction of sodium and potassium concentration dynamics, the balance of region-specific excitation and inhibition, the degree of synchrony of neuronal populations, and synaptic mechanisms such as recurrent collaterals. To understand the interplay of these dynamics, we have developed a mathematical model of a CA1 circuit consisting of conductance based neurons with intra- and extra-cellular ionic concentration dynamics. We include a two-compartment (dendrite and soma) pyramidal cell and single-compartment axo-axonic, basket, and bistratified interneurons. The synaptic strengths and extra-cellular diffusion volume of the four modeled neurons are a simplified representation of the entire CA1 network, such that the activity of any single cell in the model represent the proportion of synchronously active neurons. The synaptic connections are modeled based on estimated quantifications from experimental reports. We stimulate the network by simulating theta and gamma frequency input from CA3 and investigate the dynamics underlying seizure-like activity. Specifically, we show the sensitivity of the network as a function of the degree of synchrony of both interneurons and pyramidal cells, we assess the robustness of the model to extracellular diffusion, and we determine the sensitivity of the CA1 network to the different interneuron populations. Our results suggest experimentally testable predictions that could further elucidate the role of CA1 network dynamics in

epileptiform versus normal activity. Our model shows an optimal window of between 20-40 percent of correlated CA1 pyramidal cells for network throughput in response to CA3 input. Outside this window the network output becomes more decorrelated from CA3 input and can be prone to maintained bursting in the absence of input. This optimal window is also sensitive to correlated interneuron activity.

Disclosures: J.R. Cressman: None. **D.B. Dorman:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.14/A103

Topic: B.09. Network Interactions

Support: Max Planck Society

People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n° 302549

Title: Intracerebral recordings of slow wave and rapid eye-movement sleep in naturally sleeping pigeons

Authors: *J. VAN DER MEIJ[1], G. J. L. BECKERS[2], N. C. RATTENBORG[1];
[1]Avian Sleep Group, Max Planck Inst. For Ornithology, Seewiesen, Germany; [2]Departments of Psychology and Biol., Cognitive Neurobio. and Helmholtz Institute, Utrecht Univ., Utrecht, Netherlands

Abstract: Mammals and birds both show sleep-related slow oscillations (SO) of neuronal activity, which are thought to be involved in processing hippocampal memories at the systems level in mammals. However, unlike the mammalian hippocampus which receives input from most of the cortex, input to the avian hippocampus is restricted to olfactory and visual information, the latter coming from the hyperpallium. This variation in brain connectivity between mammals and birds may be an indication of fundamental differences in the manner of information processing at the systems level during sleep. Therefore, examining sleep-related brain activity at the systems level in birds provides us with an opportunity to test models proposed in mammals, and to reveal fundamental principles that could expand our understanding of mammalian sleep. The aim of the current study was to explore activity in the avian hyperpallium during both natural slow wave sleep (SWS) and rapid eye-movement (REM) sleep, and to compare this to activity recorded under anesthesia. We used a 32-channel silicon probe connected to a transmitter to make intracerebral recordings of the hyperpallium in naturally sleeping and isoflurane anesthetized pigeons (Columba livia) based on a within-bird design. Local field potential (LFP) recordings reveal high amplitude SO (<2Hz) across most recording

sites during natural SWS. These oscillations show diverse propagation patterns (i.e. slow waves) across the recording array. Similar results are found under anesthesia, with the exception that the SO show higher amplitude and SO activity seems more synchronized between electrode sites. In contrast, LFP recordings during REM sleep show a reduced amount of slow wave activity and lack the traveling SO seen during SWS. In conclusion, this study shows that the traveling nature of slow waves can be found in the avian hyperpallium during both natural SWS and anesthesia. As traveling slow waves have also been described in mammals, this appears to be a fundamental feature of slow wave sleep. However, simultaneous recordings of the hyperpallium and hippocampus during natural sleep are needed to evaluate the potential role that these slow waves play in processing information at a systems level in the avian brain.

Disclosures: **J. Van Der Meij:** None. **G.J.L. Beckers:** None. **N.C. Rattenborg:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.15/A104

Topic: B.09. Network Interactions

Support: PAPIIT IN216214

CONACYT 179616

CONACYT 238744

Title: Hippocampal rhythm and subfield oscillatory coupling modulation by the hypothalamic vasopressinergic magnocellular system

Authors: ***M. M. MÁRQUEZ**, H. BARRIO-ZHANG, V. S. HERNANDEZ, L. ZHANG; Nacional Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: The dorsal (dHi) and ventral (vHi) hippocampus have been considered having different roles, such as for "learning and memory" vs. "stress coping and emotional control". However, little is known concerning the oscillations coherence under different brain states between these two sub-regions. Electrophysiological studies have showed that nanomolar concentrations of [Arg8]-vasopressin (AVP) induced a prolonged increase in amplitude and slope of the evoked population response in the hippocampus. This AVP-induced potentiation of the excitatory postsynaptic potential (EPSP) persisted following removal of AVP from the perfusion medium. A pronounced effect of AVP and its metabolite AVP (4-8) was found to elicit a long-lasting enhancement of hippocampal excitability, mostly in neurons within the ventral hippocampus. Although historically the extra-hypothalamic projections of paraventricular and supraoptic nuclei were thought to be sparse, recent studies have showed that the AVP

magnocellular neurosecretory neurons possessed axon-collaterals projecting to hippocampus (Hernadez et al, 2015) and established synaptic contacts with both pyramidal and interneurons (Zhang and Hernadez, 2013). AVP innervation in hippocampus is highly heterogeneous, being the ventral and dorsal CA2 the subfields with highest density respectfully. Hence, it is interesting to evaluate the phase locking value changes between dorsal and ventral CA2 regions (dCA2 vs vCA2) under resting and activated states of the hypothalamic PVN. This investigation examined the oscillation coherence between the dorsal and ventral CA2 regions and between hypothalamic PVN and each of the previous regions, using dual-electrode *in vivo* extracellular recording and juxtacelular labeling post-recording, in rats under urethane anesthesia. PVN activation was evoked with either hypertonicity or hypovolemy, with which the hypothalamic neurohypophysial vasopresinergic pathway is predominantly up-regulated. Evolution map approach (EMA) was employed to determine the direction of information flow between dCa2 and vCA2. Preliminary results showed that salt loading increased theta oscillation / oscillation frequencies in both hypothalamic paraventricular nucleus (PVN) and the ventral CA2 region. The phase locking values of theta rhythms between PVN and the dorsal and ventral hippocampus were also increased. These data provide physiological evidences of a distinct modulatory role of the non-canonic AVP containing hypothalamic pathways on cortical oscillatory functions.

Disclosures: M.M. Márquez: None. **H. Barrio-Zhang:** None. **V.S. Hernandez:** None. **L. Zhang:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.16/A105

Topic: B.09. Network Interactions

Support: NIH Grant MH093858

NIH Grant MH102471

Title: Neural basis of at-rest band-limited fcMRI

Authors: *J. LI[1], W. J. BENTLEY[2], L. H. SNYDER[2];
[1]Washington Univ. In St Louis, Saint Louis, MO; [2]Washington Univ. in St Louis, St Louis, MO

Abstract: Functional connectivity MRI (fcMRI) studies have greatly helped our understanding of the functional organization of the human brain. fcMRI measures temporal correlations in at-rest blood oxygen level dependent signals (BOLD), which, at best, provide an indirect measure of neural activity. Though much effort has been put towards understanding the relationship between neural activity and task-related BOLD, it is unclear whether and how at-rest BOLD correlation is related to neural activity. Here we show that at-rest oxygen correlation reflects

correlated neural activity, including LFP power, slow fluctuation of raw LFP (slow LFP), single-unit spiking activity, and multi-unit spiking activity. We develop a novel method, regression-based dependency analysis, and show that oxygen correlation is largely accounted by slow LFP (72% of the within-network covariance in oxygen and 57% of the across-network covariance in oxygen), and that slow LFP correlation is accounted by spiking activity. This suggests a causal relationship, where oxygen is driven by slow LFP, which is itself driven by spiking activity. This causal link is confirmed by Granger causality analysis. In addition, we also show a feedback effect from slow LFP to spiking activity. This feedback effect is confirmed by the frequency nesting analysis, showing that the phase of slow LFP modulates the amplitude of spiking activity. The fact that spiking activity drives a delayed slow LFP change, which itself drives a feedback activity onto spiking activity could potentially lead to an oscillation in spiking activity. This oscillation could explain the band-limited correlation reported in Li et al 2014 (PNAS). In sum, our results provide conclusive support for the idea that fcMRI reflects patterns of neural activity, present significant challenges to the traditional view of neurohemodynamic coupling, and open up new avenues for exploring the functional significance of fcMRI.

Disclosures: **J. Li:** None. **W.J. Bentley:** None. **L.H. Snyder:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.17/A106

Topic: B.09. Network Interactions

Title: Influence of the stomach electrical pacemaker on spontaneous brain activity measured with fMRI

Authors: ***I. REBOLLO**, C. LEBOULLENGER, A.-D. LODEHO, C. TALLON-BAUDRY; LNC, INSERM, ENS, Paris, France

Abstract: Resting state networks (RSN) are composed of anatomically distinct brain regions whose BOLD signal at rest systematically covary. The slow (<1 Hz) correlated fluctuations in BOLD signal defining RSNs have been so far considered as consequences of factors intrinsic to the brain, such as anatomical connectivity or neural delays. However, brain activity during cognitive rest might be impacted by signals coming from other bodily organs. In particular, the brain is heavily coupled with the stomach, which acts as an autonomous pacemaker generating an electrical slow wave in the frequency range of RSNs, around 0.05 Hz. By placing electrodes over the abdomen it is possible to measure the electrical signal from the stomach pacemaker, a procedure known as Electrogastrography. We sought to investigate the influence of the stomach pacemaker on spontaneous brain dynamics by recording simultaneously the electrogastrogram and resting-state BOLD signal in 21 human participants who were asked to fixate a point during

15 minutes. We measured functional connectivity between the electrophysiological signal of the stomach and BOLD signal at each voxel. Preliminary results so far indicate that the resting-state BOLD signal in a set of areas systematically covaries with the stomach pacemaker. Those areas comprise known visceral regions, such as the insula, but also other regions such as dorsal anterior cingulate cortex, dorsal precuneus or cerebellum. These results suggest that the coupling between the stomach pacemaker and the BOLD signal at rest goes beyond areas known to directly receive inputs from the stomach and recruits in particular portions of the Saliency Network. This in turn suggest that the stomach pacemaker may play a role in shaping the large-scale organization of spontaneous brain activity.

Disclosures: I. Rebollo: None. **C. Leboullenger:** None. **A. Lodeho:** None. **C. Tallon-Baudry:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.18/A107

Topic: B.09. Network Interactions

Support: BMBF 01GQ0831

Fortüne 1331418

BMBF 16SV5838K

DFG SO932-2

EU WAY 288551

EU AIDE 645322

Title: Mapping tACS-entrained brain oscillations using magnetoencephalography (MEG)

Authors: *S. R. SOEKADAR[1], M. WITKOWSKI[1], E. GARCIA COSSIO[2], B. S. CHANDER[1], C. BRAUN[3], L. G. COHEN[4], S. E. ROBINSON[5];
[1]Applied Neurotechnology / Univ. of Tübingen, Tübingen, Germany; [2]Dept. of Artificial Intelligence, Donders Ctr. for Cognition, Radboud Univ., Nijmegen, Netherlands; [3]MEG Ctr., Univ. of Tübingen, Tübingen, Germany; [4]Human Cortical Physiol. Section, NINDS, [5]MEG Core Facility, NIMH, NIH, Bethesda, MD

Abstract: Transcranial alternating current stimulation (tACS) affects perception, memory, motor and cognitive function. The main mechanisms underlying tACS-related effects were attributed to frequency specific entrainment, i.e. phase alignment of endogenous brain oscillations to the

externally applied oscillating currents. There is, however, no established method for millimeter-precise mapping of entrained brain oscillations near and underneath the stimulator electrodes, a capability important to understand effects of tACS on brain physiology and behavior. Here we describe a new experimental protocol allowing for millimeter-precise localization and reconstruction of entrained brain oscillations underneath the stimulator electrodes, as well as reliable reconstruction of neuromagnetic oscillations across all physiological frequency bands. Reliability of the protocol was tested in a phantom study (study 1) and a group of healthy human volunteers engaging in a motor task (study 2). Source space analysis was applied to data recorded in absence and during amplitude-modulated tACS delivered at different frequencies (11Hz, 23Hz). In study 1, spatial precision of localizing phase-locked activity between an oscillation dipole and tACS was assessed by evaluating drop in phase-lock value (PLV) as a function of distance from a known fixed dipole's position. In study 2, tACS-entrained brain oscillations were mapped on the individual's brain anatomy and results tested for consistency. Use of this approach can open a new window to investigate mechanisms and effects of stimulation protocols that entrain brain oscillations.

Disclosures: **S.R. Soekadar:** None. **M. Witkowski:** None. **E. Garcia Cossio:** None. **B.S. Chander:** None. **C. Braun:** None. **L.G. Cohen:** None. **S.E. Robinson:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.19/A108

Topic: B.09. Network Interactions

Support: MEXT KAKENHI Grant Number 26120512

Title: Frequency dependent entrainment of spontaneous Ca transients by extracellular AC electric fields in CA1 pyramidal neurons of rat hippocampal slices

Authors: *I. KATO[1], H. MIYAKAWA[2], M. INOUE[2], T. AONISHI[1];
[1]Tokyo Inst. of Technol., Yokohama Kanagawa, Japan; [2]Lab. of Cell. Neurobio., Tokyo university of Pharm. and Life Sci., Hachioji, Japan

Abstract: In central nervous systems, extracellular electric fields are oscillating due possibly to the activities of neural cells. We hypothesized that oscillating extracellular electric fields modulate activity of neuronal population providing a mean to correlate or synchronize neuronal activities. It has been shown that various types of ion channels distributed along the dendrites and endow the dendrites with the capability of generating dendritic spikes including Na spikes, Ca spikes and NMDA spikes. We think it conceivable that generation of dendritic spikes play important roles in sensing extracellular electric field or enhancing ephaptic interactions by

providing current source to generate electric fields. To test the former idea, we developed a method to monitor local Ca transients associated with dendritic spikes in the dendrites of a population of neurons in rat hippocampal slices using spinning disk confocal microscopy and multi-cell dye loading techniques. In a condition where the dendrites of CA1 pyramidal neurons show spontaneous activity because of 50 μM 4-aminopyridine added to external medium and adjusted extracellular potassium concentration (5.5mM), we previously reported that the timing of spontaneously occurring Ca transients in the tufts of the apical dendrites of CA1 pyramidal neurons would be entrained to sub-threshold (1~20mV/mm) 4Hz electric fields applied parallel to the somato-dendritic axis of neurons. Here, we examined the frequency dependence of this entrainment by applying weak (6.5mV/mm) sinusoidal electric fields ranging from 1-16Hz (n=4). We found that within this range, the entrainment was most evident at 2Hz and less evident at other frequencies. Our results support the idea that dendritic action potentials may play important roles in detecting electric oscillating fields.

Disclosures: **I. Kato:** None. **H. Miyakawa:** None. **M. Inoue:** None. **T. Aonishi:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.20/B1

Topic: B.09. Network Interactions

Support: NFR of Korea Grant No. 2013057789

Title: Effect of inter-modular connection on fast sparse synchronization in clustered small-world networks

Authors: ***S.-Y. KIM**, W. LIM;
Inst. of Computat. Neuroscience, Dept. of Sci. Educ., Inst. of Computat. Neurosci., Daegu, Korea, Republic of

Abstract: We consider a clustered network with small-world sub-networks of inhibitory fast spiking Izhikevich interneurons, and investigate the effect of inter-modular connection on emergence of fast sparsely synchronized rhythms by varying both the inter-modular coupling strength J_inter and the average number of inter-modular links per interneuron M_syn^(inter). In contrast to the case of non-modular networks, modular and global sparsely synchronized states are found. For the case of modular sparse synchronization the population behavior reveals the clustering structure, because the intra-modular dynamics of sub-networks make some mismatching. On the other hand, in the case of global sparse synchronization, the population behavior is globally identical, independently of the cluster structure, because intra-modular dynamics of sub-networks make perfect matching. We use a realistic cross-correlation

modularity measure, representing the matching-degree between the instantaneous sub-population spike rates of the sub-networks, and examine whether the sparse synchronization is global or modular. Depending on its magnitude, the inter-modular coupling strength J_inter seems to play "dual" roles for the pacing between spikes in each sub-network. For large J_inter, due to strong inhibition it plays a destructive role to "spoil" the pacing between sparse spikes, while for small J_inter it plays a constructive role to "favor" the pacing between spikes. Through competition between the constructive and destructive roles of J_inter, there exists an intermediate optimal J_inter at which the pacing degree between spikes becomes maximal. In contrast, the average number of inter-modular links per interneuron M_syn^(inter) seems to play a role just to "favor" global communication between sub-networks. With increasing M_syn^(inter), the degree of effectiveness of global communication increases monotonically. Furthermore, we employ the realistic whole- and sub-population order parameters, based on the instantaneous whole- and sub-population spike rates, to determine the threshold values for the synchronization-unsynchronization transition in the whole- and sub-populations, and the degrees of the global and modular synchronization are also measured in terms of the realistic statistical-mechanical whole- and sub-population spiking measures defined by considering both the occupation and the pacing degrees of the spikes. It is expected that our results have important implications for the role of the brain plasticity which refers to the brain's ability to change its structure and function by modifying the strength or efficacy of synaptic transmission.

Disclosures: **S. Kim:** None. **W. Lim:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.21/B2

Topic: B.09. Network Interactions

Title: *In vivo* characterization of hippocampal theta and gamma spontaneous oscillations in familial Alzheimer's disease mouse models based on mutant presenilin-2

Authors: ***R. FONTANA**[1], M. RUBEGA[2], G. SPARACINO[2], C. FASOLATO[3], S. VASSANELLI[3];
[1]Dept. of Biomed. Sci., Univ. of Padua, Padova, Italy; [2]Dept. of Information Engin., [3]Dept. of Biomed. Sci., Univ. of Padua, Padua, Italy

Abstract: Neuronal network oscillations, measured by electroencephalography (EEG) or local field potential (LFP), have long been linked to several cognitive activities in both humans and rodents. In particular, hippocampal oscillations in the theta and gamma frequency ranges are considered to underlay important computational functions, e.g. providing key reference signals for temporal encoding of information. Indeed, it has been observed that the interaction between

the phase of hippocampal theta oscillations and the amplitude of hippocampal gamma oscillations assessed by phase-amplitude cross-frequency coupling (CFC) analysis plays a pivotal role in learning performance. Alzheimer's disease (AD) is a highly diffuse and severe brain pathology characterized by progressive impairments in cognition and memory. One major histological feature of AD is the accumulation of senile plaques, consisting of amyloid-beta (Aβ) peptides. Aβ derives from the sequential processing of the amyloid precursor protein (APP) by β and γ secretases. Familial AD (FAD) is caused by different missense mutations affecting the genes encoding for APP and presenilin (PS1 and PS2), i.e. respectively the substrate and the core component of γ secretase. Recent evidence suggests an alteration of theta and gamma rhythms in AD. Furthermore, the phase-amplitude CFC in these two frequency bands is impaired in *ex vivo* and *in vivo* AD mouse models. At present, therapies attempting to revert or stop AD are poorly effective at least partially owing to a late diagnosis. Biomarkers for an early diagnosis of the pathology, i.e. before the emergence of the cognitive deficits, are critical for improving the efficacy of current and future strategies to contrast the progression of the disease. The aim of this work is to identify early EEG markers in FAD mouse models characterized by precocious calcium dysregulation in addition to Aβ load and/or plaque deposition, i.e. the homozygous mouse lines PS2.30H and B6.152H, that express respectively the human PS2-N141I mutation alone or in combination with the human APP Swedish mutation. Within this frame we have recorded spontaneous LFP activity from the hippocampus of urethane-anesthetized mice and characterized theta and gamma oscillations together with their phase-amplitude CFC - before and after amyloid plaque deposition. Preliminary data suggest an increased power in the slow gamma range (25-45 Hz) in the PS2.30H and B6.152H lines along with an altered theta-gamma CFC in the B6.152H line in both 3 and 6 months old female mice with respect to age- and sex-matched wild-type mice.

Disclosures: **R. Fontana:** None. **M. Rubega:** None. **G. Sparacino:** None. **C. Fasolato:** None. **S. Vassanelli:** None.

Poster

293. Oscillations and Synchrony: Other I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 293.22/B3

Topic: F.01. Human Cognition and Behavior

Support: Alfred P. Sloan Foundation Research Fellowship

UC San Diego Qualcomm Institute Calit2 Strategic Research Opportunities program

Title: Spike-field coupling does not imply spike-spike coupling

Authors: *E. PETERSON, B. VOYTEK;
Cognitive Sci., U.C. San Diego, San Diego, CA

Abstract: The origin and function of oscillatory activity remains a major outstanding question in neuroscience. One prominent hypothesis for the functional role of gamma oscillations is 'communication through coherence'. This theory posits that regional coherence enhances communication by increasing the precision of spike timing, i.e. spike-spike coupling. The focus on coherence has lead to computational investigations of already oscillating populations. While important in establishing coherence as useful for communications, and in showing how information flow is maximized when coherence between oscillating pairs is maximized, these studies skip over a basic question: is spike-time precision enhanced by the onset and amplitude of gamma oscillations? By definition, oscillating neural populations have repeating periods of decreased firing. If all else is held equal, these periods of relative silence would mean a decrease in information flow. As firing declines so does information. If oscillations increase information flow, they must alter spiking to overcome these 'silent costs'. Keeping with the idea that oscillations alter spike timing, and using Hodgkin-Huxley neurons in classic excitatory-inhibitory configurations, we simulated the effect of gamma onset and amplitude on spike precision and on information flow. Our simulations suggest a much larger range of parameters can generate gamma oscillations, compared to only a narrow range of parameters that can actually increase precision and information transmission in excitatory neurons. From a theoretical perspective, our results suggest the 'communication through coherence' hypothesis may require fairly stringent biophysical constraints to function as proposed. When aggregating over all models, gamma power does not statistically predict spike precision, nor does a change to spike-field coupling imply a change in spike-spike coupling. In sum these results suggest gamma oscillations, when driven solely by excitatory-inhibitory interactions, reflect mostly silent periods rather than the spike-time shifting necessary for enhanced precision.

Disclosures: **E. Peterson:** None. **B. Voytek:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.01/B4

Topic: B.10. Intrinsic Membrane Properties

Support: NIH

NSERC

QEII-GSST

SciNet HPC Consortium

Title: Investigating spiking resonance in computational models of oriens-lacunosum/moleculare (O-LM) hippocampal interneurons with dendritic synaptic inputs

Authors: *V. SEKULIC[1,2], J. J. LAWRENCE[4], F. K. SKINNER[1,3,2];
[1]Toronto Western Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada; [2]Physiol., [3]Med. (Neurology), Univ. of Toronto, Toronto, ON, Canada; [4]Pharmacol. and Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: The theta rhythm (4-12Hz) is a prominent network oscillation observed in the mammalian hippocampus and is correlated with spatial navigation and mnemonic processing. Inhibitory interneurons of the hippocampus fire action potentials at specific phases of the theta rhythm, pointing to distinct functional roles of interneurons in shaping this rhythmic activity. One prominently studied interneuron type is the oriens/lacunosum moleculare (O-LM) cell, which provides direct feedback inhibition and regulation of pyramidal cell activity in the CA1 region. O-LM cells express the hyperpolarization-activated, mixed-cation current (Ih) and, *in vitro*, demonstrate spontaneous firing at theta frequencies that is impaired upon blockade of Ih. Recent work using dynamic clamp (Kispersky et al. 2012) has shown that in the presence of theta frequency-modulated artificial synaptic inputs, O-LM cells exhibit a spiking resonance at theta that is not dependent on Ih. Due to the somatic injection limitation of dynamic clamp, the study could not examine the potential contributions of dendritic conductances and the integration of dendritically-located synaptic inputs. Here, we used previously developed multi-compartment computational models of O-LM cells to begin to address these issues. The models were extracted from our previous ensemble modeling work that showed that dendritic expression of Ih supports appropriate O-LM cell firing. We selected models with dendritic Ih and inserted excitatory and inhibitory synaptic inputs onto the dendritic tree of the models. The synapses were randomly activated at rates according to a Poisson distribution. Modulation of the input at various frequencies resulted in changes in the power spectral density of the model spiking activity. Our results indicate that models with synaptic inputs spread across the dendritic tree express enhanced resonant firing at theta frequencies compared to models with synaptic inputs in the soma only. Thus, the presence of dendritic Ih may interact with synaptic inputs onto dendrites to enhance spiking output in O-LM cells at theta frequencies. This implies that theta-timed inputs onto O-LM cells, such as from the medial septum, may preferentially target their dendrites in order to maximally recruit O-LM cell firing at theta frequencies. Investigating these network interactions are of critical importance for elucidating O-LM cell contributions to theta rhythm activity in the CA1 microcircuit.

Disclosures: **V. Sekulic:** None. **J.J. Lawrence:** None. **F.K. Skinner:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.02/B5

Topic: B.10. Intrinsic Membrane Properties

Support: NSERC Discovery Grant

CIHR Operating Grant

Title: Predicting cell-type specific active properties by developing multi-compartment models using databases and electrophysiological feature constraints: Application to interneuron specific 3 (IS3) cells in the hippocampus

Authors: *A. T. GUET-MCCREIGHT[1,2], O. CAMIRÉ[4,5], L. TOPOLNIK[4,5], F. K. SKINNER[1,2,3];
[1]Toronto Western Res. Inst., Toronto, ON, Canada; [2]Dept. of Physiol., [3]Dept. of Med. (Neurology), Univ. of Toronto, Toronto, ON, Canada; [4]Ctr. de Recherche de l'Institut Universitaire en Santé Mentale de Québec, Québec City, QC, Canada; [5]Dept. of Biochemistry, Microbiology and Bioinformatics, Univ. Laval, Québec City, QC, Canada

Abstract: In hippocampus, interneuron-specific type 3 (IS3) cells make GABAergic synapses onto specific types of interneurons, including oriens lacunosum-moleculare (OLM) cells. These synapses possess relatively weak synaptic transmission properties, but despite this, IS3 cells are able to control OLM cell firing patterns (Tyan et al, 2014). As such, we assume that IS3 spikes need to be initiated with minimal synaptic inputs so that synaptic summation is more likely to occur. Morphological and synaptic aspects of IS3 cells are being examined, but what type, how much and where voltage-gated channels (VGCs) are present on IS3 cells has not been determined. Other hippocampal interneuron types are known to have high densities of VGCs on their dendrites. High densities of some types of VGCs (e.g. sodium) along IS3 dendrites may serve to facilitate spike initiation. Using a combination of the NEURON software environment for running simulations and a MATLAB toolbox called PANDORA (Günay et al, 2009) for experimental and simulation analysis, we used both automation and hand tuning to develop IS3 cell models. Data on the active and passive membrane properties of IS3 cells was acquired experimentally, and two-photon calcium imaging was used to assess the spread of back-propagating action potentials in IS3 dendrites, which were seen in dendritic branches as far as 150 μm away from the soma. Using this data as a target reference, we generated databases of multi-compartment models, each one possessing unique combinations of channel types & conductance values. Models that did not capture the features that were seen in experimental recordings were eliminated from each database. We then identified the remaining models in each database whose measurements most closely resembled those seen in experimental traces. Once these models were identified, we computed the minimal excitatory synaptic input necessary to elicit a somatic spike when the synapse was applied at different points along the dendritic arbor. Given the present correspondence with data, our models predict relative conductance balances of different channel types in IS3 cells. Our models also predict that transient sodium and fast delayed rectifier potassium VGCs are present in the proximal dendrites and that the synaptic input necessary to elicit somatic spikes is minimized when A-type potassium channels are

restricted to the soma. Moving forward, our models can serve as a basis for understanding the functional roles of IS3 cells in the hippocampus, a central structure in memory formation.

Disclosures: A.T. Guet-Mccreight: None. **O. Camiré:** None. **L. Topolnik:** None. **F.K. Skinner:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.03/B6

Topic: B.10. Intrinsic Membrane Properties

Support: Schram Foundation

Lichtenberg Professorship Award (Volkswagen Foun- dation

German Research Foundation Grant BA1582/2-1

Research Unit FOR2143, funded by the German Research Foundation

Title: Dendritic integration in dentate gyrus parvalbumin expressing perisoma inhibiting interneurons

Authors: *C. ELGUETA, M. BARTOS;
Freiburg Univ., Freiburg Im Breisgau, Germany

Abstract: Parvalbumin (PV^+) positive GABAergic perisoma-inhibiting interneurons (PIIs) enable cortical circuits to perform complex operations by providing precisely timed feed-forward or feedback inhibition into principal cells. In despite of their central role during neuronal network computations, little is known regarding how signals are processed in their dendritic trees. Using a combination of single cell voltage sensitive dye imaging (VSDI) and glutamate uncaging in acute slice preparations of the rat dentate gyrus, we have characterized the integrative properties of PV^+ PII dendrites. First we determined speed and attenuation of back-propagating action potentials. We observe a monotonic decline in size of APs as a function of distance when traversing from the soma along both, basal and apical dendrites. In agreement with the low density of sodium conductances, blocking voltage-gated sodium channels did not changed AP attenuation. In line with the high density of Kv3-type potassium channels, blocking them with 4AP had a facilitating effect on action potential backpropagation. Second, a combination of VSDI and glutamate uncaging revealed strong attenuation and deceleration of locally evoked excitatory postsynaptic potentials, suggesting that PII dendrites favor precision and speed during signal integration. Moreover, irrespective of the spatial distribution of the evoked localized EPSPs, summation of excitatory signals at the soma was always sub-linear. Third, we examine how $GABA_A$ receptor-mediated inhibition modulates the EPSPs integration

in PII dendrites by analyzing the effect of localized GABA uncaging using DPNI or RUBI-GABA onto size and shape of EPSPs evoked by glutamate microiontophoresis (miEPSPs). We observe that distal (off-path) inhibition is more effective than proximal (on-path) inhibition in reducing the amplitude of miEPSPs recorded at the soma, although this depended in the size of the excitatory potential and the chloride reversal potential at the uncage locations. In summary, our data suggest than proximal $GABA_A$ receptor-mediated inhibition is better suited to control action potential generation while distal inhibitory inputs favor the regulation of size and shape of excitatory signals.

Disclosures: C. Elgueta: None. **M. Bartos:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.04/B7

Topic: B.10. Intrinsic Membrane Properties

Support: Ministry of Science, ICT and Future Planning of Korea (13-01-HRSS-03 and 14-RS-02)

Title: Characterization of dendritic processing for signal propagation in model primary neurons

Authors: *H. KIM;
Daegu Gyeongbuk Inst. of Sci. & Technol., Daegu, Korea, Republic of

Abstract: Primary neurons in the central nervous system show very distinctive morphology of their dendrites that contain thousands of synaptic contacts as well as a variety of voltage-gated ion channels. The goal of this study is to investigate how cell type-specific dendritic structure is related to dendritic processing for signal propagation in primary neurons. We first anatomically reconstructed model primary neurons: spinal motor neuron, hippocampal pyramidal neuron, neocortical pyramidal neuron, and cerebellar Purkinje neuron. Dendritic signal propagation was then characterized by calculating the voltage attenuation factor between the soma and all points in the dendrites as a function of path length from the soma. We found: 1) that the voltage attenuation data could be well captured with a single fitting equation as a function of distance for both (soma-to-dendrite and dendrite-to-soma) signal propagation directions, and 2) that all anatomically reconstructed models of primary neurons display a similar asymmetric signal propagation in their dendrites: voltage attenuation was much severer for the direction from the dendrites and the soma compared to the opposite direction from the soma to the dendrites. These results may serve to bridge the gap between realistic and reduced neuronal models in a basis of physical distance along the dendrites.

Disclosures: H. Kim: None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.05/B8

Topic: B.10. Intrinsic Membrane Properties

Support: UBACyt-20020100100962

APCyT PICT 2010-0606

Title: Graded boosting of synaptic signals by low threshold voltage activated calcium conductance

Authors: M. CARBÓ-TANO[1], *L. SZCZUPAK[2,1];
[1]IFIByNE UBA-CONICET, Buenos Aires, Argentina; [2]Univ. de Buenos Aires, Buenos Aires, Argentina

Abstract: Estilo predeterminado; The neuritic tree is the cellular domain at which neurons integrate and process synaptic inputs. In the past decades it became clear that dendrites do not rely purely on their passive membrane properties but are supplied with voltage-activated conductances that affect their processing capabilities. Among these conductances, low threshold voltage-activated calcium conductances (LT-VACCs) play a substantial role in shaping the biophysical attributes of neurites suggesting their involvement in synaptic processing. However, it is not known to what extent the activation of these conductances shapes the synaptic responses. Here we have investigated how LT-VACCs affect synaptic integration in a premotor nonspiking (NS) neuron of the leech nervous system. These cells exhibit an extensive neuritic tree, do not fire Na^+-dependent spikes but express a LT-VACC that was sensitive to 250 μM Ni^{2+} and 100 μM NNC 55-0396 (NNC). Calcium imaging studies showed that this LT-VACC is distributed throughout the main branches and is activated by synaptic responses evoked by stimulation of pressure sensitive (P) neurons. The resulting Ca^{2+} signals were a graded function of the electrophysiological synaptic responses and spread throughout the neuritic tree with no major attenuation. Thus in NS neurons synaptically-evoked Ca^{2+} signals are a graded function of synaptic inputs that spread globally. NNC decreased these synaptic responses and abolished the concomitant widespread Ca^{2+} signals. Coherent with the interpretation that the LT-VACC amplified signals at the postsynaptic level, this conductance also amplified the responses of NS neurons to direct injection of sinusoidal current. Synaptic amplification thus is achieved via a positive feedback in which depolarizing signals activate a LT-VACC that, in turn, boosts these signals. The results presented here indicate that LT-VACC amplified the responses to synaptic inputs. This boosting was achieved in a graded manner and the amplified signal preserved a linear relationship with the firing frequency of the presynaptic neuron. The wide distribution of LT-VACC could support the active propagation of depolarizing signals, turning the complex NS neuritic tree into a relatively compact electrical compartment.

Disclosures: **M. Carbó-Tano:** None. **L. Szczupak:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.06/B9

Topic: B.10. Intrinsic Membrane Properties

Title: Continuous gradients of gene expression underlie prominent CA1 pyramidal neuron variability

Authors: ***M. S. CEMBROWSKI**, J. L. BACHMAN, L. WANG, K. SUGINO, B. SHIELDS, N. SPRUSTON;
Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Tissue and organ function has been conventionally understood in terms of the interactions among discrete and functionally homogeneous cell types; however, this discretization approach has traditionally proved difficult in neuroscience. Here, we systematically evaluated the extent to which a discrete cell-type framework characterizes a canonical cell population of the mammalian forebrain - hippocampal CA1 pyramidal cells (CA1 PCs). Using next-generation RNA sequencing (RNA-seq) of subsets of CA1 pyramidal cells, we found differences in CA1 PCs to be markedly greater than detected using previous methods, with hundreds of genes being differentially expressed across the long (dorsal-ventral) axis of CA1. We show that CA1 PC transcriptional identity emerges from a spectrum of gene expression gradients, resulting in the CA1 PC population existing as a diverse continuum of cells, rather than conforming to discrete cell types. These transcriptional differences extend to both proteomic and functional levels. Surprisingly, the overall difference within CA1 PCs across the long axis rivals that of differences across pyramidal cell classes, suggesting that these within-class differences may produce prominent spatially variable computation in CA1 PCs. This work demonstrates an unexpected amount of variability present within a canonical and narrowly defined neuronal population, and suggests that within-class neuronal variation may be an important and feature of neuronal circuits.

Disclosures: **M.S. Cembrowski:** None. **J.L. Bachman:** None. **L. Wang:** None. **K. Sugino:** None. **B. Shields:** None. **N. Spruston:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.07/B10

Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant NS085729

Title: Localized synaptically activated sodium signals in hippocampal pyramidal neurons show both AMPA and NMDA receptor components

Authors: *K. MIYAZAKI, W. N. ROSS;
New York Med. Col., Valhalla, NY

Abstract: Na+ imaging can reveal information about the location and properties of synaptically activated glutamate receptors and voltage gated Na+ channels. It can indicate events due to AMPA receptor activation, which are not revealed by calcium imaging since these receptors are impermeant to Ca2+ in most pyramidal neurons. We developed a system to simultaneously detect both [Na+]i and [Ca2+]i changes at high speed (250 Hz in each channel). [Na+]i changes were detected with either SBFI or ANG-2. Both Na+ and Ca2+ indicators were loaded together with a patch electrode on the soma. Fluorescence changes from dendrites on neurons in hippocampal slices were detected with a high speed CCD camera. Trains of 3-5 synaptic stimuli at 50 Hz at subthreshold intensities reliably evoked [Na+]i increases in a localized region (3-10 μm) of the dendrites. Careful examination suggested that the origin of the signals was more localized and that some of the spread was due to Na+ diffusion. These signals persisted in the presence of CPP, suggesting that the signals were due to Na+ entry through AMPA receptors. Consistent with this interpretation no [Ca2+]i changes were detected although Ca2+ signals from bAPs were reliably detected at the same locations. In most cases the Na+ signals did not begin until the 3rd or later synaptic response, although occasionally signals were detected from the 1st stimulus. Experiments with bAPs showed that the system detected Na+ signals with only a few ms delay from the spike, demonstrating that the synaptic delay was real. With stronger stimulation, or more stimuli (but still subthreshold) a later, APV sensitive signal was detected, suggesting an NMDA receptor component. This signal persisted longer than the AMPA component. As previously reported, bAPs, by themselves, evoked very small [Na+]i changes in the dendrites. In some trials bAPs, either evoked by the synaptic train or by intrasomatic stimulation, generated a sharp increase in [Na+]i at the synaptic site at the time of the spike, suggesting that the spike triggered Na+ entry. However, in many other trials the spike triggered no additional signal even when the synaptic train evoked a subthreshold Na+ signal. Therefore, the importance of a bAP for coincident activation of NMDA receptors is not clear. Stronger stimulation, especially in the basal dendrites, evoked much larger [Na+]i and [Ca2+]i signals that were detected over a wider region. These signals had the characteristics of an NMDA spike. Most of the Ca2+ signal was blocked by APV, while most of the Na+ signal remained, suggesting that even following a regenerative NMDA receptor event most Na+ entry is through AMPA receptors at pyramidal neurons synapses.

Disclosures: K. Miyazaki: None. **W.N. ross:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.08/B11

Topic: B.10. Intrinsic Membrane Properties

Support: European Research Council (ERC-2010-StG-260590)

Deutsche Forschungsgemeinschaft (Exc 257 NeuroCure and DFG-FOR-1341 BaCoFun)

Helmholtz foundation

Title: Two-photon subcellular optogenetic stimulation of layer 2/3 cortical pyramidal neurons *in vivo* during network activity

Authors: *L. FERRARESE[1,2], J. F. A. POULET[1,2];
[1]Max Delbrueck Ctr. Berlin-Buch, Berlin, Germany; [2]Neurosci. Res. Ctr. and Cluster of Excellence NeuroCure, Charité-Universitätsmedizin, Berlin, Germany

Abstract: *In vivo*, synaptic transmission and integration in cortical neurons occurs on a background of ongoing, depolarized, subthreshold activity during active brain states. To investigate subthreshold integration *in vivo* we made targeted somatic whole-cell recordings of L2/3 pyramidal neurons expressing Channelrhodopsin2 in primary somatosensory cortex of urethane anaesthetised mice that oscillate between hyperpolarised, quiescent Downstates and active depolarized Upstates. We then mimicked synaptic input with brief (10 ms) two-photon laser pulses that triggered reliable subthreshold depolarizing potentials at different subcellular targets. At the soma, optogenetic potentials was significantly reduced in amplitude during Upstates to 82% of the Downstate amplitude and showed an increase in half-width (from 20 ms to 34 ms). Similar changes in amplitude and half-width were seen during periods of depolarizing current injection during Downstates, Isopotential of Upstate. The broadening of the optogenetic potential was linked to an increase in somatic input resistance during Upstates. Depolarising potentials recorded at the soma could also be evoked on dendritic branches up to 140 μm from the somatic recording site. Dendritic stimulation triggered smaller amplitude, longer latency responses than somatic stimulation and showed differing degrees of state dependent modulation. *In vivo* subcellular optogenetic stimulation provides a new approach to study subthreshold integration during active cortical states and suggests that the postsynaptic input site is a key determinant of state dependent modulation in response amplitude.

Disclosures: L. Ferrarese: None. **J.F.A. Poulet:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.09/B12

Topic: B.10. Intrinsic Membrane Properties

Support: ISF 845/12

Einstein Foundation

NIPI

Title: *In vivo* adrenergic modulation of dendritic HCN channels in layer 5 pyramidal neurons

Authors: *M. LONDON, C. LABARRERA MØNSTED;
Edmond and Lily Safra Ctr. for Brain Sci. and Life Sci. Inst., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The hyperpolarization activated cation current Ih generated through HCN channels is an important current shaping the synaptic integration properties in cortical pyramidal neurons of layer 5. The density of HCN channels is non-uniform over the dendritic tree of these cells and is concentrated in the distal apical tree. In addition it has been suggested that Ih is a key player in persistent activity of pyramidal neurons during working memory tasks and that its modulation through cAMP by Guanfacine (an alpha 2A receptor agonist) enhances this effect. To better understand the interaction between the dendritic distribution of Ih and the effects of its modulation we performed whole-cell recordings from layer 5 pyramidal neurons in awake head restrained mice. We first established that Ih can be blocked under these conditions using ZD 7288. We then show that Ih is modulated by application of Guanfacine but surprisingly that this modulation has little effect on some membrane properties such as action potential threshold and resting membrane potential. We hypothesized that due to its dendritic distribution, modulation of Ih by Guanfacine will affect the coupling between the somatic compartment and the apical tuft. To test this hypothesis we used the critical frequency protocol. We demonstrated that application of either ZD 7288 or Guanfacine significantly reduces the threshold for Ca nonlinear activity in the distal dendrites. We conclude that adrenergic modulation of Ih in the apical dendrites of cortical pyramidal neurons has an effect on Ca nonlinear integration in the apical tuft and on the interaction between soma and dendrites. This effect implies that adrenergic modulation of Ih may serve as a regulator of the coupling between feedforward input arriving at the basal dendritic tree of L5 neurons and feedback input arriving at the apical tuft.

Disclosures: **M. London:** None. **C. Labarrera Mønsted:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.10/B13

Topic: B.10. Intrinsic Membrane Properties

Support: European Research Council under the European Community's Seventh Framework Programme (FP7), ERC-StG #261114

National Multiple Sclerosis Society (RG 4924A1/1)

Title: Functional role of coupling axons to dendrites in layer 5 pyramidal neurons

Authors: *M. S. HAMADA, M. H. KOLE;
Axonal Signaling, Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: The axon initial segment (AIS) is a unique domain of axons in the nervous system converting the graded synaptic inputs into axonal action potential (AP) rate and temporal codes. While most studies depict the axon origin at the soma base, more than hundred years of anatomical studies from many different cell types indicate that axons also regularly branch from dendrites. In order to understand the functional implications of axon coupling to dendrites, we recorded from thick-tufted layer 5 pyramidal neurons using whole-cell patch-clamp and correlated their structural features with the functional properties (n = 40). Neurons were subsequently stained for AIS-specific anchoring protein markers and biocytin-streptavidin and scanned with confocal laser-scanning microscopy. Approximately ~40% of thick-tufted L5 neurons possessed axons with a basal dendrite origin. As expected, the AIS onset in these axo-dendritic neurons was significantly further away from the soma edge (~10 μm; $P < 0.0001$). The AIS length was, however, not different. Morphological analyses of 3D reconstructions revealed a lower dendritic branch order complexity and thinner apical dendritic diameter in axo-dendritic neurons compared to axo-somatic neurons (6.4 vs. 8.2 μm; $P < 0.00001$). Interestingly, the apical dendrite diameter linearly and negatively correlated with the AIS onset ($r = -0.7148$, $P < 0.0001$, $n = 40$). Analysis of AP properties showed that axo-dendritic neurons had a ~3 mV lower voltage threshold ($P = 0.02$) and a higher firing rate at steady-state current injection (23 Hz at 0.8 nA; $P = 0.022$) which was confirmed in computational modelling to scale with the experimentally observed differences in axon onset location. The combined empirical and modelling data revealed that differential axon onsets enable tuning and normalization of synaptic integration in the face of varying geometries of the dendritic trees.

Disclosures: **M.S. Hamada:** None. **M.H. Kole:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.11/B14

Topic: B.10. Intrinsic Membrane Properties

Title: Active dendritic integration in L5 pyramidal neurons forms a conjunctive sensorimotor representation that contributes to learning

Authors: *G. NATTAR RANGANATHAN[1], N.-L. XU[2], J. C. MAGEE[1];
[1]Howard Hughes Med. Inst., Ashburn, VA; [2]Inst. of Neurosci., Shanghai Inst. for Biol. Sciences, Chinese Acad. of Sci., Shanghai, China

Abstract: Rodents scan their immediate facial environment by active whisker sensing. This process requires combining of sensory and motor inputs in order to adapt whisking behavior to changing sensory conditions. Layer 5 (L5) pyramidal neurons in the rodent barrel cortex preferentially receive sensory and motor inputs onto spatially separated regions of their dendritic arbor. Active dendritic mechanisms in these neurons have been shown to enable the integration of coincident but spatially separated sensory and motor inputs during a whisker-dependent object localization task. The global dendritic plateau potentials that mediate this integration have been observed to have joint feature selectivity to both sensory and motor information. Here we present that the somatic output of L5 pyramidal neurons also carries a similar conjunctive representation of sensory and motor information. Responses of individual neurons are dependent on the strength of whisker touch measured from changes in curvature, but also modulated by the whisker position in which touch occurred. L5 neuronal activity at the population level indicates a mixed representation of joint feature selectivity, with varying degrees of modulation at a wide range of whisker positions across the population. Preferential silencing of the dendritic plateau potentials through optogenetic methods interferes with learning in a sensory cue driven motor refinement task. These data indicate that active input integration in the distal apical dendrites of L5 pyramidal neurons mediates a conjunctive sensorimotor representation in the L5 population. Disruption of this representation by silencing active dendritic integration results in the impairment of learned refinement in whisking behavior. Experiments are being conducted to measure the precise effects of optogenetic silencing on the population representation of L5 pyramidal neurons during the sensorimotor task.

Disclosures: G. Nattar Ranganathan: None. **N. Xu:** None. **J.C. Magee:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.12/B15

Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant: RO1 NS044163

VA-BLR&D Merit Review: 821-MRNB-24218

Title: Integration of synaptic input during active firing in the L5 pyramidal neurons of mouse motor cortex

Authors: *N. C. DEMBROW[1,2], G. S. NEWKIRK[1], W. SPAIN[1,2];
[1]Physiol. & Biophysics, Univ. of Washington, Seattle, WA; [2]Neurol., VA Epilepsy Ctr. for Excellence, Seattle, WA

Abstract: It is evident from *in vivo* recordings that cortical pyramidal neurons (PNs) transition between periods of actively firing action potentials and relative quiescence. Such changes in PN activity result from changes in the spatial and temporal patterns of their synaptic inputs. How synaptic inputs are integrated depends upon both the intrinsic properties of the target neuron as well as their temporal and spatial distribution. These features are difficult to control in the awake, intact brain but can be studied in the *ex vivo* slice preparation. To do this, we recorded from L5 PNs in motor cortex in Thy1-h YFP mice using whole-cell patch clamp combined with 2-photon glutamate uncaging. Individual spines within basal and proximal apical oblique branches of L5 neurons were activated by uncaging glutamate with brief, focused pulses of light (200 μs at 720 nm). Single spine responses recorded at the soma were variable in size (0.52 ± 0.35 mV; 109 spines from 10 neurons). As has been described in other neuron types, activating multiple spines upon a single branch with near temporal synchrony (2000 Hz) triggered somatic EPSPs that were greater than the linear sum of individual inputs. We observed supralinear responses in 7/9 Thy1-h neurons (47.5 ± 11.1% increase in 7 cells), suggesting active currents in the dendrites enhanced the measured somatic response. To test how the activation of individual spines altered the firing frequency, we stimulated single spines while driving the neuron to fire with step current injection (10-20 Hz). Single spine responses elicited a 5-20% increase in the instantaneous firing frequency (0.75 ± 0.35 Hz, across 71 responses). While the increase in the instantaneous firing frequency depended upon EPSP size (r^2 = 0.36182, slope = 1.73 Hz/mV), in some cases even small (<0.3 mV) EPSPs triggered 1-2 Hz increases in firing rate. When current noise (exponentially filtered noise, tau = 5 ms, SD of 50 pA) was included in the somatic current injection, the effects of single spine inputs became less apparent. We next tested whether activation of multiple spines on a single branch (>10) with near synchrony drove more robust increases in the firing rate. Supralinear branch integration is inhibited by backpropagating action potentials in some cell types, and thus such nonlinearities may not persist in the active firing regime. The near synchronous multi spine activation caused large elevation of firing rate when paired with either step or noisy somatic current injections (40.69 ± 17 Hz, 8 neurons). Combined our data shows that during the active firing regime both single and spatiotemporally clustered spine activation can influence neuronal output.

Disclosures: N.C. Dembrow: None. **G.S. Newkirk:** None. **W. Spain:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.13/B16

Topic: B.10. Intrinsic Membrane Properties

Support: Natural Sciences and Engineering Research Council of Canada

NIMH grant MH048432

NIMH grant MH094839

Title: Dendritic nonlinearities are tuned to fast synaptic inward currents

Authors: B. KALMBACH[1], *R. A. GRAY[1], D. JOHNSTON[1], E. COOK[2];
[1]Ctr. for Learning and Memory, The Univ. of Texas At Austin, Austin, TX; [2]Physiol., McGill Univ., Montreal, QC, Canada

Abstract: We applied a systems-identification approach to quantitatively describe the nonlinear behavior of dendrites. Dendritic membrane potentials (whole-cell patch) were recorded from L5 pyramidal neurons in mouse prefrontal cortex in response to either small (subthreshold) or large (suprathreshold) 10 kHz white-noise current injections. Convolving the small current injection with a linear filter fully accounted for the dendritic membrane potential (median variance accounted for or VAF = 99%). The filters were relatively fast, with an initial exponential-like decay (median 1/e decay = 2 ms) followed by an under-shoot that produced a theta resonance. The large suprathreshold injections produced both somatic action potentials (spikes) and dendritic spike-like nonlinearities. The large injections produced the same linear filters as the small injections, but they accounted for less of the membrane potential because of the additional nonlinearities (median VAF = 94%). Deconvolution was used to isolate the nonlinear components from the linear response. The results of the deconvolution revealed nonlinear inward current spikes (median rate of ~20 spikes/sec), that when added to the white-noise current injection, accounted for 100% of the dendritic membrane potential. In individual neurons, these dendritic spikes varied in amplitude by over a factor of 10 and were abolished by TTX, but not APV. We modeled the isolated dendritic spikes using a second linear spike-filter followed by a static nonlinearity. When convolved with the large stimulus, the spike-filter/nonlinear cascade described both the dendritic spike amplitude (median VAF = 85%) and the time that each dendritic spike occurred. In comparison to the filters that described the linear membrane potential, the dendritic spike-filters were much faster (median 1/e decay = 0.5 ms). Our results show that the dendritic membrane potential in response to a white-noise current injection is well described by two components: 1) A filter that captures the linear membrane response and, 2) A second faster spike-filter followed by a static nonlinearity that captures the nonlinear dendritic spikes. The shape of the spike-filter suggests that dendritic nonlinearities are tuned to the rise time of fast inward current events such as AMPA-mediated synaptic inputs.

Disclosures: **B. Kalmbach:** None. **R.A. Gray:** None. **D. Johnston:** None. **E. Cook:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.14/B17

Topic: B.10. Intrinsic Membrane Properties

Title: The interplay between synaptic and nonsynaptic activity

Authors: Y. BUSKILA[1], *J. C. TAPSON[2], J. MORLEY[3], A. VAN SCHAIK[1];
[1]The MARCS Inst., Univ. of Western Sydney, Bankstown, Australia; [2]Director's Unit, Univ. of Western Sydney, Penrith, Australia; [3]Sch. of Med., Univ. of Western Sydney, Cambelltown, Australia

Abstract: Neurons in the brain use action potentials (spikes) to communicate with each other. In the postsynaptic cell, these spikes lead to synaptic signals (EPSPs) that propagate through the dendrites and are finally summed at the site of spike initiation. Cortical and hippocampal neurons are sensitive to the timing of their synaptic inputs as they can synchronize their firing on a millisecond time scale, and follow rapid stimulus fluctuations with high temporal precision. In recent years, several studies have reported large variability of spike propagation delays in networks of neurons processing these signals, and it is thought that these delays are modulated to enhance signal integration and thus optimize synaptic inputs. Moreover, computational studies refer to the spike propagation delays as storage capacity units, and predict that the neuronal networks use these delays to time signals and encode information. In order to understand how synaptic, and intrinsic nonsynaptic, properties interact and integrate to optimize neuronal output, we have established an STDP protocol using photoactivation of Channelrhodopsin-2 containing neurons, and recorded synaptic activity arriving from multiple sites along the dendritic tree of cortical and hippocampal pyramidal neurons. Our findings indicate a correlation between the synaptic modifications and propagation velocity along the dendritic branch, suggesting that nonsynaptic modifications along the dendrites are accompanying the synaptic changes.

Disclosures: **Y. Buskila:** None. **J.C. Tapson:** None. **J. Morley:** None. **A. Van Schaik:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.15/B18

Topic: B.10. Intrinsic Membrane Properties

Title: HippoSeq: a cell class- and region-specific RNA-seq atlas of excitatory neurons in the hippocampus

Authors: *N. P. SPRUSTON[1], L. WANG[2], K. SUGINO[2], B. SHIELDS[2], M. S. CEMBROWSKI[2];
[1]Scientific Program Director and Lab. Head, Ashburn, VA; [2]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Understanding gene expression in specific neuronal populations can provide important insight into cellular identity and functionality. Here, we used next-generation RNA sequencing (RNA-seq) to produce a quantitative, whole-genome transcriptional atlas of gene expression spanning every excitatory neuronal population in the hippocampus; namely, granule cells and mossy cells of the dentate gyrus, and pyramidal cells of areas CA3, CA2, and CA1. Moreover, for the granule and pyramidal cells comprising the classical trisynaptic loop, we profiled transcriptomes at both dorsal and ventral poles, producing a cell class- and region-specific transcriptional atlas for these canonical populations. This atlas defines the transcriptional properties and identities of lesser known cell types (mossy cells and CA2 pyramidal cells), reveals unexpectedly large variations in the trisynaptic loop across the dorsal-ventral axis, and ultimately presents a cohesive, large-scale characterization of the transcriptional rules of the hippocampus. We have created an interactive website - HippoSeq - that enables user-friendly analysis and visualization of these data. These transcriptional profiles and associated website will act as a quantitative, whole-genome roadmap for further dissection of the molecules, cells, and circuits in the hippocampus.

Disclosures: **N.P. Spruston:** None. **L. Wang:** None. **K. Sugino:** None. **B. Shields:** None. **M.S. Cembrowski:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.16/B19

Topic: B.10. Intrinsic Membrane Properties

Support: Young Investigators Grant (Faculty of medicine Goethe-University to P.J.)

BMBF grant (Germany-USA Collaboration in Computational Neuroscience to P.J., No. 01GQ1203A)

BMBF grant (No. 01GQ1406 – Bernstein Award 2013 to H.C.)

International Max Planck Research School for Neural Circuits, Frankfurt

Title: A consistent electrophysiological model of dentate granule cells based on pharmacology to study adult-born neurons

Authors: *M. BEINING[1,2,3], L. A. MONGIAT[4], S. SCHWARZACHER[3], H. CUNTZ[1,2], P. JEDLICKA[3];
[1]Ernst Strüngmann Inst. (ESI) For Neurosci., Frankfurt, Germany; [2]Frankfurt Inst. for Advanced Studies, Frankfurt, Germany; [3]Inst. of Clin. Neuroanatomy, Neurosci. Ctr., Goethe Univ. Frankfurt, Frankfurt, Germany; [4]Inst. de Investigación en Biodiversidad y Medioambiente, CONICET-Universidad Nacional Comahue, San Carlos de Bariloche, Argentina

Abstract: Granule cells in the dentate gyrus (DGCs) are continuously generated in the adult mammal brain. Their integration into the hippocampal network has been linked to important brain functions such as pattern separation and spatial memory formation. Interestingly, DGCs show a critical time window, in which they express higher excitability, synaptic plasticity and excitation-to-inhibition ratio, each phenomenon linked to specific protein expression or connectivity. A data-driven single-cell compartmental model with realistic morphology and ion channel composition would provide an excellent tool to analyze the effect of all these changes in intrinsic and synaptic properties on neuronal computation. However, existing active compartmental models of DGCs have limited predictive capability since they were constructed *ad hoc* in order to replicate mostly one single experiment. Here we present a novel active model for mature DGCs based on raw electrophysiological traces combined with pharmacology in mice (*1*). Our new model includes a corrected calcium buffer model, an inward rectifier potassium (Kir) channel, several isoforms of voltage-gated potassium channels and modified distributions of BK, SK as well as L-, T-, N-type calcium channels, consistent with the known DGC ion channel composition. The resulting model reproduces experimental electrophysiology in mature DGCs. Furthermore, the calcium dynamics, as well as spiking characteristics such as afterhyperpolarization, depolarizing afterpotential and spiking adaption are in a realistic range indicating that the DGC compartmental model is consistent across a variety of experimental paradigms. The model can now be adapted to simulate synaptic integration and plasticity in adult-born DGCs. Our preliminary simulations suggest, that NMDA subunit composition alone might not suffice to fully explain the increased capability for synaptic plasticity during the critical time window (*2*). In summary, we created a novel active DGC compartmental model, which is consistent in ion channel composition and electrophysiology, enabling us to make valuable predictions about single-cell computation in mature and adult-born DGCs. 1) L. a Mongiat, M. S. Espósito, G. Lombardi, A. F. Schinder, *PLoS One*. **4**, e5320 (2009). 2) S. Ge, C.-H. Yang, K.-S. Hsu, G.-L. Ming, H. Song, *Neuron*. **54**, 559–66 (2007).

Disclosures: M. Beining: None. **L.A. Mongiat:** None. **S. Schwarzacher:** None. **H. Cuntz:** None. **P. Jedlicka:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.17/B20

Topic: B.10. Intrinsic Membrane Properties

Support: Boehringer Ingelheim Fonds

Wellcome Trust WT094077MA

ERC AdG 250345

Title: Synaptic input patterns underlying dendritic computation *in vivo*

Authors: L. GOETZ[1], M. R. GROEN[1], *A. ROTH[2,1], M. HAUSSER[1];
[1]Wolfson Inst. for Biomed. Res., [2]Univ. Col. London, London, United Kingdom

Abstract: The computations performed by an individual neuron critically depend on the spatiotemporal patterns of synaptic input it receives onto its dendrites. While the detailed synaptic input patterns are still largely inaccessible to experiment, the ability to use dendritic patch-clamp recording to measure sensory-evoked local dendritic spikes *in vivo* (Smith et al., Nature 2013) provides valuable constraints on possible spatiotemporal patterns of synaptic input. Furthermore, extensive experimental data on the biophysical properties of dendrites, the statistics of sensory responses of neurons, as well as the characteristics and function of synaptic inputs *in vivo* have recently become available. Using these experimental results, we have developed a detailed active biophysical model of a neocortical layer 2/3 pyramidal neuron to explore which spatiotemporal patterns of synaptic inputs can drive the observed neuronal input-output transformations. Our model generates fast dendritic spikes heterogeneous in amplitude, time course and spatial extent as observed *in vivo* (Smith et al., 2013), which are abolished by removing NMDA receptors. We used spike-triggered averaging to identify the spatiotemporal synaptic input patterns preceding dendritic spikes. Dendritic spikes are preferentially triggered by excitatory synapses which are spatially and temporally clustered on the local branch and neighbouring branches. The temporal patterns of excitatory synaptic input preceding spikes are stereotyped and tend to be surprisingly sparse. Notably, only some dendritic spikes are effective in evoking action potential output, while others remain local. We used cluster analysis to differentiate between these cases and establish global conditions under which specific spatiotemporal patterns of synaptic input can influence neuronal output. Thus, our simulations provide a mechanistic explanation for neuronal computations observed *in vivo*, for example how output tuning to stimulus features arises from both tuned and untuned inputs. To summarize, we derive spatial and temporal rules for how synaptic inputs drive dendritic spikes. These results suggest that synaptic input patterns may be optimized to locally exploit dendritic excitability to shape the input-output relation of the neuron.

Disclosures: **L. Goetz:** None. **M.R. Groen:** None. **A. Roth:** None. **M. Hausser:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.18/B21

Topic: B.10. Intrinsic Membrane Properties

Support: SNF Grant PP00P3_128415

Title: Dendritic serotoninergic neuromodulation in the anterior cingulate cortex alleviates neuropathic pain

Authors: M. SANTELLO, A. BISCO, *T. NEVIAN;
Univ. of Bern, Bern, Switzerland

Abstract: Neuropathic pain is caused by long-term modifications of neuronal function in the peripheral nervous system, the spinal cord, and supraspinal areas. Although functional changes in the forebrain are thought to contribute to the development of persistent pain, their significance and precise subcellular nature remain unexplored. Using somatic and dendritic whole-cell patch-clamp recordings from neurons in the anterior cingulate cortex, we discovered that sciatic nerve injury caused a dysfunction of hyperpolarization-activated cyclic nucleotide-regulated (HCN) channels in the dendrites of layer 5 pyramidal neurons resulting in enhanced integration of excitatory postsynaptic inputs and increased neuronal firing. Consistent with a HCN channel hypofunction, resonance frequency was lower in CCI neurons. The pathology was caused by an activity-dependent decrease in HCN channel density in the apical dendrite. Specific activation of the serotonin receptor type 7 (5-HT7R) alleviated the lesion-induced pathology by increasing HCN channel function, restoring normal dendritic integration, and reducing mechanical pain hypersensitivity in nerve-injured animals *in vivo*. Thus, serotoninergic neuromodulation at the forebrain level can reverse the dendritic dysfunction induced by neuropathic pain and may represent a potential therapeutical target.

Disclosures: **M. Santello:** None. **A. Bisco:** None. **T. Nevian:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.19/B22

Topic: B.10. Intrinsic Membrane Properties

Support: NHMRC 1085708

NHMRC 1063533

Title: Local dendritic modulation by multimodal sensory input

Authors: *L. M. PALMER[1], M. E. LARKUM[2];
[1]Florey Inst. of Neurosci. and Mental Hlth., Univ. of Melbourne, Melbourne, Australia;
[2]Humboldt Univ., Berlin, Germany

Abstract: In the living animal, sensory systems are generally not stimulated in isolation but are instead activated collectively. In response to multimodal sensory stimulation, the dendrites of pyramidal neurons in the primary somatosensory cortex receive both feedforward input from the thalamus and feedback input from other cortical areas. The influence of this feedback multimodal input on dendritic function is input specific and largely unknown. We investigated dendritic activity in the hindpaw somatosensory cortex during contralateral hindpaw stimulation alone and during activation of additional sensory-evoked feedback input. Using both single-cell electrophysiology and dendritic 2-photon calcium imaging *in vivo*, we show that sensory input from the stimulation of a second sense, in this case forepaw stimulation, had different effects on the distal and proximal dendrites of layer 2/3 pyramidal neurons. Forepaw stimulation caused a decrease in the Ca2+ activity of tuft dendrites whereas proximal dendrites had an increase in synaptic input. Taken together, this multimodal input onto layer 2/3 pyramidal neurons led to a balancing of sensory information resulting in no change to the evoked firing rate. These results not only illustrate the counterbalancing interaction of multimodal input in cortical neurons but it also highlights the complexity of local dendritic activity in sensory perception.

Disclosures: L.M. Palmer: None. **M.E. Larkum:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.20/B23

Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant 1P01NS079419

Charles A. King Trust

NIH Grant P41GM103712

Dept of Energy CSGF

Title: Control of spatially patterned gene expression in dendrites

Authors: *A. H. WILLIAMS[1], C. O'DONNELL[2], T. SEJNOWSKI[2], E. MARDER[3], T. O'LEARY[3];
[1]UC San Diego, La Jolla, CA; [2]Salk Inst. for Biol. Studies, La Jolla, CA; [3]Brandeis Univ., Waltham, MA

Abstract: The subcellular spatial distribution of ion channels and synaptic plasticity-related proteins is dynamic, nonuniform, and critical for determining the electrical properties of neurons. How neurons control these expression patterns is unknown, but is believed to involve microtubular-based transport. Using mathematical analysis and numerical simulations, we show how transport can support these phenomena in complex neuron morphologies. While the results we derive are general to any cell type, we examine CA1 pyramidal morphology as a case study, specifically examining: 1) HCN and K ion channel gradients in dendrites and 2) spatial expression of plasticity-related proteins such as Arc. We compare two non-exclusive models for achieving spatial expression: local versus global control. For the local-control class, we derive simple rules that relate the local transport rate along neurites to the global steady-state distribution of cargo, and illustrate how this rule can be encoded by a second-messenger molecule, such as Ca2+. We identify two strategies that precisely achieve any desired expression profile. The first distributes cargo uniformly along the microtubules, and locally captures cargo in proportion to the target expression level. The second distributes cargo non-uniformly to match the target expression pattern and locally releases cargo at a uniform rate. There is experimental evidence for both of these strategies [1,2]. We also considered a second class of models based on global control, in which the steady-state distribution of cargo along the microtubules is not achieved. In this case, the distribution of cargo might be controlled by tuning the microtubular transport kinetics to match the rate of local release/capture of cargo. A major difference between these two classes of models is that the first can be locally tuned, while the second must be engineered from a global level. We suggest that local mechanisms might be useful to produce flexible trafficking, while global mechanisms provide efficient solutions for coarse, rapid transport. We highlight experiments that might disambiguate between these two cases.
References: [1] Kim S, Martin KC (2015). eLife. 4:e04158 [2] Steward O, Farris S, Pirbhoy PS, Darnell J, Van Driesche SJ (2015). Front Mol Neurosci. 7:101

Disclosures: A.H. Williams: None. **C. O'Donnell:** None. **T. Sejnowski:** None. **E. Marder:** None. **T. O'Leary:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.21/B24

Topic: B.10. Intrinsic Membrane Properties

Support: NIH grant NS32405 (W.G.R)

NIH grant NS087708 (S.R.)

Title: Active dendrites and differential calcium channel distribution allow Golgi cells to independently regulate dendritic processing and spontaneous firing

Authors: *S. RUDOLPH[1], C. HULL[2], W. G. REGEHR[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Neurobio., Duke Univ. Sch. of Med., Durham, NC

Abstract: Golgi cells (GoCs) are spontaneously active interneurons that continuously release GABA to inhibit granule cells and thereby control the excitability of the input layer of the cerebellar cortex. Previous studies concluded that GoC dendrites lack voltage-gated sodium, calcium and potassium channels. As a result of their passive properties, synaptic inputs onto distal dendrites are strongly attenuated. Here, we use a combination of whole-cell recording and dendritic calcium imaging to re-examine dendritic properties and calcium-dependent regulation of firing in GoCs. We find that GoC dendrites contain voltage-gated sodium channels, and support back-propagating action potentials that open voltage-gated calcium channels throughout the dendritic tree. We also find that calcium channel subtypes are differentially distributed and functionally specialized. R-type and T-type calcium channels are absent from the soma, but present in distal dendrites. T-type calcium channels promote burst firing and boost synaptic inputs onto distal dendrites. Conversely, N-type calcium channels are primarily present near the soma where they regulate spontaneous firing through their close association with calcium-activated potassium channels. We find that GoCs have a low calcium buffer capacity, and as a result a modest calcium channel density produces large dendritic calcium signals. In light of our finding that GoCs dendrites are active, the manner in which GoCs process synaptic inputs from granule cells must be reevaluated. The differential distribution of calcium channels allows dendritic T-type channels to boost synaptic inputs and N-type channels near the soma to regulate spontaneous firing.

Disclosures: **S. Rudolph:** None. **C. Hull:** None. **W.G. Regehr:** None.

Poster

294. Dendritic Excitability and Synaptic Integration

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 294.22/B25

Topic: B.10. Intrinsic Membrane Properties

Support: DE018661

Title: Electrophysiological properties of nodes of ranvier revealed by patch-clamp recordings from intact afferent nerve fibers of mammals

Authors: *J. GU, H. KANDA, W.-P. CHANG, J. LING;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Electrophysiological properties of nodes of ranvier are important for saltatory conduction along myelinated nerve fibers and have pathological implications. Much of what we know about the electrophysiological properties of nodes of ranvier came from previous studies performed on large axons of the invertebrates, and due to technical challenge patch-clamp recordings have not been applied to nodes of ranvier in an intact nerve fiber. In the present study, we developed a whole-cell patch-clamp recording method to study electrophysiological properties of nodes of ranvier of intact rat trigeminal afferent nerve fibers. We observed two major currents at nodes of ranvier in responses to voltage steps, a large voltage-activated fast inward current and a large leak current. The fast inward current was mediated by voltage-gated Na^+ channels since it was completely blocked by TTX. The large leak current was resistant to both TTX and intracellular Cs^+ and was most likely due to axonal conductance. Action potentials could be elicited at nodes of ranvier by depolarizing currents and they were completely blocked by TTX. Action potentials were not significantly altered when 135 mM Cs^+ was included as a voltage-gated K^+ channel blocker in the recording internal solution. In addition to the two major currents, we also observed a small tail current which could be inhibited by linopirdine, a KCNQ channel blocker. Linopirdine reduced resting membrane potentials (less negative) and action potential rhoebase. We studied propagation of action potentials through nodes of ranvier by electric stimulation at a distant site (1 cm) from the recorded node of ranvier. Nodes of ranviers could well follow electric stimulation at the frequency up to 100 Hz with full-sized action potentials. At the stimulation frequency above 100 Hz, action potential firing at nodes of ranvier showed frequency-dependent reduction of their action potential amplitudes. Taken together, our results provide new insights into electrophysiological properties of nodes of ranvier of mammals.

Disclosures: J. Gu: None. **H. Kanda:** None. **W. Chang:** None. **J. Ling:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.01/B26

Topic: B.11. Glial Mechanisms

Support: CIHR

Title: Expression of APP and its processing enzymes in glial cells of APP transgenic mice with or without Npc1 protein

Authors: *A. SASSE;
Univ. of Alberta, Edmonton, AB, Canada

Abstract: Evidence suggests that increased levels of amyloid beta (Aβ peptides derived from amyloid precursor protein (APP) contribute to the development of Alzheimer's disease (AD). The regions primarily affected in AD brains are hippocampus and cortex, whereas striatum and cerebellum are relatively spared. Although neurons are considered to be the major source of Aβ in the brain, the activated astrocytes associated with neuritic plaques, the key neuropathological hallmark of AD brains, have also been shown to accumulate Aβ Since cholesterol has been shown to influence Aβ production, it is of interest to determine whether accumulation of cholesterol within endosomal-lysosomal system, the major site of Aβ production, can influence levels and/or processing of APP. To address this issue we used mutant APP transgenic (APP-Tg) mice, mice lacking Neimann Pick Type C1 (Npc1-null) protein required for intracellular cholesterol transport and our recently developed bigenic ANPC mice that overexpress mutant human APP in absence of Npc1 protein. The results obtained so far indicate that APP and its processing enzymes involved in Aβ production such as β-secretase BACE1 and four components of γ-secretase complex (PS1, nicastrin, Pen2 and APH1) are expressed in a subset of reactive astrocytes in ANPC, APP-Tg and Npc1-null mice but not in age-matched wild-type control mice. The relative number of astrocytes expressing APP and its processing enzymes appear more in ANPC>APP-Tg>Npc1-null mice. These results indicate that reactive astrocytes may have an important role in the generation of Aβ-peptides in AD-related pathology. Additionally, accumulation of cholesterol within endosomal-lysosomal system may influence APP levels/processing in the activated astrocytes.

Disclosures: A. Sasse: None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.02/B27

Topic: B.11. Glial Mechanisms

Support: NIMH grant R01 MH067715

Title: Astrocytes control synapses formation and pruning: is FGF signaling pathway involved?

Authors: *S. SCUDERI[1], H. E. STEVENS[1,4], S. TOMASI[1], F. DRAGO[5], F. M. VACCARINO[1,2,3];
[1]Child Study Ctr., [2]Dept. of Neurobio, [3]Kavli Inst. for Neurosci, Yale Univ., New Haven, CT; [4]Carver Col. of Med., Univ. of Iowa, Iowa City, IA; [5]Biomed. and Biotechnological Sci., Univ. of Catania, Catania, Italy

Abstract: Astrocytes are emerging as key elements in many aspects of brain development, function and disease. In particular, there is evidence for dynamic neuroglial interactions at the so-called "tripartite synapse". Astrocytic processes intimately interact with synapses, but little is known about the role of astrocytes in the control of synapse number and plasticity in the developing and adult brain. Previous data link fibroblast growth factor (FGF) signaling to astrocyte differentiation, morphology and functions. Based on this, we hypothesized that under normal conditions astrocytes utilize FGF receptors 1 and 2 (FGFR1/2) and other adhesion molecules located at their lamellar processes to interact with and modulate the number of synaptic connections. To test this, we induced an astrocyte-specific loss of Fgfr1/2 in the early postnatal period by using transgenic mouse lines. Brain tissue was collected from three month old mice with induced Fgfr1/2 loss (FgfR1/2 iKO) and control mice; synaptic markers (VGLUT/PSD95, VGAT/Gephyrin) and adhesion molecules were assessed using immunohistochemistry, western blot analysis and Real Time PCR. Increased expression of vesicular transporters for both excitatory and inhibitory synapses (VGLUT1 and VGAT) was observed at both mRNA and protein levels in FgfR2 iKO brains compared to control. These perturbations were not randomly distributed in the cortex, but were more pronounced in the upper cortical layers. Furthermore, in mutant mice, an altered expression of cell adhesion molecules expressed at astrocyte-neuron membrane contacts was observed. Interestingly, the FgfR iKO mice also showed locomotor hyperactivity and impairments in cognitive and social behavior compared to control littermates. Our findings support the idea that astrocyte Fgf signaling controls synaptic remodeling in early postnatal development, which may be a process that may exert a profound impact on neural network function and behavior.

Disclosures: S. Scuderi: None. **H.E. Stevens:** None. **S. Tomasi:** None. **F. Drago:** None. **F.M. Vaccarino:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.03/B28

Topic: B.11. Glial Mechanisms

Support: NIH/NINDS research grant R01NS088058

Title: Regulatory role of proinflammatory cytokines in the expression of aromatase in reactive astrocytes

Authors: *J. WANG[1], R. K. VADLAMUDI[2], D. BRANN[1];
[1]DNRM, Georgia Regents Univ., Augusta, GA; [2]Obstetrics and Gynecology, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Numerous studies have shown that the steroid hormone, 17β-Estradiol (E2) is not only a gonadal-derived hormone but also a brain-derived neuroprotective factor. So far little is known regarding how the local production of E2 in the forebrain is controlled. The expression of brain aromatase, the enzyme that catalyzes the conversion of testosterone into E2, is generally found to be restricted to certain neuronal populations under normal conditions. We recently reported that de novo expression of aromatase in astrocytes can be induced by global cerebral ischemia and exerts anti-inflammatory and neuroprotective actions in the rodent hippocampus. It is important to note that induction of high aromatase expression in the injured brain was accompanied by the astrocyte reactivation. To investigate the regulatory mechanism that underlie the induction of aromatase in reactive astrocytes after injury, we examined the expression of aromatase in primary astrocytes cultured from male and female neonatal mouse brain under different treatments (oxygen and glucose deprivation [OGD], extracellular ATP, and Lipopolysaccharide [LPS]). These treatments are all well known as triggers of astrocyte reactivation, and mimic the *in vivo* events of ischemia, cell damage and inflammation after brain injury. The results of the study revealed that LPS induced a significant increase of aromatase expression in astrocytes in an acute phase (2-4 hour post treatment). OGD also increased aromatase expression in astrocytes, but the effect occurred later (after 24 hours). These findings support a potential role for injury-induced inflammation in the induction of aromatase expression in reactive astrocytes. We next examined the effect of proinflammatory cytokines (IL-1βand IL-6) upon induction of aromatase in cultured astrocytes. The results revealed that both cytokines were able to enhance aromatase expression as efficiently as LPS. While the mechanism of the cytokine up-regulation of aromatase is unclear, the up-regulation of aromatase by the cytokines was associated with an elevated expression of the CCAAT/enhancer binding protein beta (C/EBPβ). This is intriguing as C/EBPβ is a transcription factor implicated to mediate proinflammatory cytokine actions, and the aromatase gene is known to contain 16 C/EBPβ binding sites. Taken as a whole, our findings suggest that ischemic injury-induced release of proinflammatory cytokines can drive induction of aromatase expression in astrocytes, and that this effect may involve mediation by the transcriptional factor, C/EBPβ. (Supported by NIH/NINDS research grant R01NS088058)

Disclosures: **J. Wang:** None. **R.K. Vadlamudi:** None. **D. Brann:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.04/B29

Topic: B.11. Glial Mechanisms

Title: The novel state of astrocyte for inducing long-term memory with contexture fear conditioning test

Authors: *M. CHOI, H.-S. KIM;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Astrocytes are the most abundant cell type in the central nervous system (CNS). It is well-known that astrocytes maintain ion homeostasis in the extracellular space and also release immune response factors. However, recent studies regarding the tripartite synapse suggest that astrocytes may directly modulate synapses by releasing neuroactive molecules such as glutamate, ATP, and D-serine, or by uptaking neurotransmitters from synaptic cleft. Such evidence suggest that astrocytes may play a key role in long-term depression (LTD) and in long-term potentiation (LTP). Astrocytes have two different states, which are the resting state and the reactive state. The reactive state is shown in inflammation and neuronal disorders, such as Alzheimer's disease (AD). Morphological changes in the reactive state include increase in thickness and number of processes, and increase in size of soma. Molecular changes also occur, such as an increase in expression of glial fibrillary acidic protein (GFAP). These morphological and molecular changes may be in line of functional changes. However, the specific morphological and molecular changes in the event of long-term memory formation have not been explored. In this study, we induced long-term memory by subjecting mice to the contextual fear conditioning test (CFC), and check for changes of astrocytes that correspond to the reactive state. Results show that in the memory induction stage, astrocytes exhibit a unique condition which differs from the resting or reactive state. Further studies may be conducted to elucidate this novel state, and possibly link changes in this state with cognitive dysfunction or neurodegenerative diseases such as AD.

Disclosures: **M. Choi:** A. Employment/Salary (full or part-time):; Brain Korea 21 plus. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Korea Healthcare Technology R&D project A111230, National Research Foundation of Korea 2011-0021866. **H. Kim:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.05/B30

Topic: B.11. Glial Mechanisms

Support: Euro-Trans-BIO In-HEALTH

BMBF # 031B0010B

Title: Cultured astrocytes exhibit high-frequency voltage oscillations

Authors: *S. THEISS[1], W. FLEISCHER[2], J. SLOTTA[2], A. SCHNITZLER[2];
[1]Inst. of Clin. Neurosci., [2]Inst. of Clin. Neurosci. and Med. Psychology, Univ. of Duesseldorf, Duesseldorf, Germany

Abstract: High frequency oscillations (HFOs) occur in brain tissue both under physiological and pathological conditions, and have been recorded *in vitro*, in animals and in humans. In the healthy brain they likely support memory related functions, while in epilepsy patients they have been associated with seizure onset zones. In an *in vitro* model of dissociated neuronal networks cultured on microelectrode arrays (MEAs), localized HFOs were recorded in response to brief electrical stimuli, and persisted under blockage of action potentials and synaptic transmission. In order to examine the role of astrocytes in the generation of HFOs, we cultured cortical astrocytes from P0 rats on microelectrode arrays (square grid of 8x8 Ti/TiN electrodes, diameter 30 µm, spacing 200 µm), and applied ten single biphasic rectangular voltage pulses (800 mV, 200 µs/phase) to a subset of electrodes. Recordings were performed in low osmolarity HEPES-buffered saline solution similar to Neurobasal medium at pH 7.4. Stimulus-averaged spectral power of oscillations was calculated from the signal's time-frequency representation. In response to the electrical stimulus pulses, astrocytes exhibited strong extracellular voltage fluctuations in a broad frequency spectrum (100 to 600 Hz) that could last several seconds. These aperiodic HFOs were constrained to the stimulated electrode. The voltage-gated calcium channel antagonist cilnidipine dose-dependently decreased oscillation power. HFOs were dependent on intracellular calcium, since the Ca2+-chelator BAPTA-AM suppressed HFOs as well as substitution of extracellular Ca2+ by Ba2+. Incubation with bafilomycin A1 showed that vesicular release of transmitters played only an inferior role in the emergence of HFOs. Gap junctions and volume-regulated anionic channels had just as little functional impact, which was demonstrated by the addition of carbenoxolone (100 µM) and NPPB (100 µM). Hyperpolarization with low potassium in the extracellular solution (2 mM) dramatically raised oscillation power. A similar effect was seen when we added extra sodium (+50 mM) or if we replaced it with NMDG+ (50 mM). The purinergic receptor antagonist PPADS suppressed oscillation power, while the agonist ATP (100 µM) had only an increasing effect when the bath solution pH was slightly lowered to pH 7.2. We conclude that astrocytic voltage oscillations were triggered by activation of voltage-gated calcium channels and driven by a downstream influx of cations through channels that are permeable for large ions such as NMDG+. Most likely candidates are subtypes of pore-forming P2X channels with a low affinity for ATP.

Disclosures: S. Theiss: None. **W. Fleischer:** None. **J. Slotta:** None. **A. Schnitzler:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.06/B31

Topic: B.11. Glial Mechanisms

Support: NIH NIDCD R01 DC010844

NIH P30-DC005983

Title: Acoustic trauma-induced strial vascular degeneration is associated with motile myofibroblastic PCs

Authors: *X. SHI, Dr., Z. HOU, J. CAI, X. WANG, J. ZHANG;
Oregon Hearing Res. Ctr., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Stria vascularis capillary destruction and regression after loud sound trauma has long been observed, but the mechanism underlying the pathology has not been understood. In this study, we found a proportion of strial pericytes (PCs) had converted to the phenotype of motile myofibroblastic PCs at approximately one week after loud sound stimulation. Using both *in vivo* animal based and *in vitro* primary cell line based models, the strial PCs transition is highly associated with increased transforming growth factor beta 1 (TGF-β1). The transition to myofibroblastic PCs is significantly reduced when TGF-β1 is suppressed with the TGF-β1 receptor blocker, SB431542, or blocked using a recombined adenovirus carrying a PEDF gene (ad-PEDF) to up-regulate pigment epithelium derived factor (PEDF). Reduced capillary density following acoustic trauma was correlated with PC phenotype changes. With newly established *in vitro* three-dimensional cell-based co-culture models, we demonstrated that PCs are essential for maintaining normal vascular architecture and stability. The architecture becomes markedly thicker with branches less finely articulated with changes in PC phenotype. The results suggest that loud sound triggered emergence of motile myofibroblastic PCs may be one of causes for noise-induced capillary degeneration.

Disclosures: **X. Shi:** None. **Z. Hou:** None. **J. Cai:** None. **X. Wang:** None. **J. Zhang:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.07/B32

Topic: B.11. Glial Mechanisms

Support: the Ministry of Science and Technology, Taiwan

Title: The death mechanism of extracellular acidosis on neonatal rat astrocytes

Authors: *Y. WANG[1,2], Y.-F. CHEN[3], Y.-M. LEUNG[4];
[2]Dept. of Chinese Pharmaceut. Sci. and Chinese Med. Resources, [3]Dept. of Pharmacol., [4]Dept. of Physiol., [1]China Med. Univ., Taichung, Taiwan

Abstract: During brain ischemia, acidosis, resulting from the metabolism elements of the anaerobic glycolysis, is a critical consequence of causing neuronal cell death. Astrocytes serve many important roles, including nourishing and providing mechanical support for the neurons, metabolizing neurotransmitters and secreting gliotransmitters to modulate neurotransmission. Numerous evidence indicates insult in these astrocyte functions during brain ischemia can crucially influence neuron survival. However, much less is known about the fate of astrocyte in acidosis. The purpose of our study was to investigate cell death mechanisms of rat astrocytes in acidosis per se (independent of hypoxia). We isolated cortical astrocyte cultures from 1 to 2-day-old Sprague Dawley rats. Before each experiment, primary astrocytes were incubated in different acidic pH culture media for indicated time courses. MTT assay was used to determine cell viability. In addition to elucidate the signaling pathway acidosis-induced activation, we used fura-2 as the Ca2+ -sensitive fluorescent dye for performing microfluorimetric measurement of cytosolic Ca2+ concentration, immunoblotting for analysis of individual proteins, and ApoSENSOR ADP/ATP ratio assay kit for detecting ADP/ATP ratio. Our results showed that astrocytes suffered from cell death after challenge by acidic pH (6.8, 6.0, 5.0) for 2-24 h. Exposure to acidic pH caused intracellular Ca2+ release and extracellular Ca2+ influx; however, abrogation of cytosolic Ca2+ elevation by BAPTA did not prevent acidic pH-induced cell death. Acidic pH caused p38 MAPK activation, Akt inhibition, mitochondrial depolarization, decreased reactive oxygen species (ROS) formation and increased ADP/ATP ratio. Cyclosporin A, which binds to cyclophilin D and hence inhibits mitochondrial permeability transition pore (PTP), could prevent acidity-induced cell death. In conclusion, these findings suggest acidosis caused astrocyte death not by cytosolic Ca2+ overload but by p38 MAPK activation and mitochondrial PTP opening.

Disclosures: **Y. Wang:** None. **Y. Chen:** None. **Y. Leung:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.08/B33

Topic: B.11. Glial Mechanisms

Support: Swiss National Science Foundation

Title: Modulation by metformin and AICAR of astrocytic glucose metabolism

Authors: *I. ALLAMAN[1], G. GRENNINGLOH[1], C. BARRIERE BORGIONI[1], P. J. MAGISTRETTI[1,2];
[1]EPFL/Brain Mind Inst., Lausanne, Switzerland; [2]Div. of Biol. and Envrn. Sci. and Engin., KAUST, Thuwal, Saudi Arabia

Abstract: Metformin is a widely used drug for the treatment of type 2 diabetes. While it increases peripheral glucose uptake and utilization in patients, its actions on the central nervous system are less characterized. In the present study, we first aimed to determine the effects of metformin on astrocytes, a cell type playing a key role in cerebral energy metabolism. Using primary cultures of mouse cortical astrocytes, we observed that metformin at 500 uM stimulates 3H-2-deoxyglucose uptake (i.e. glucose utilization) in a time-dependent manner, with a maximal effect after 4 hours of treatment (+239% versus control values). A more detailed characterization of metformin's effects showed that stimulation of glucose utilization is accompanied by an increase in lactate release (+160% versus control values) as well as a decrease in glycogen levels (26% of control values). In contrast to the observed increase of glycolytic metabolism, oxidative metabolism of glucose was not significantly modified by metformin as determined by measurement of total CO2 production using 14C(U)-glucose as substrate (93% of control values). As some of metformin's peripheral metabolic effects are known to be mediated by AMP-activated kinase (AMPK), we evaluated the impact of AMPK activator AICAR on astrocytic glucose utilization. Surprisingly we observed that AICAR was not only unable to reproduce the stimulatory action of metformin but even presented an opposite effect, decreasing glucose utilization (by 75%). This latter observation suggested that the above described metabolic effects of metformin were independent of AMPK activation. Further supporting such view, the stimulation of glucose utilization induced by metformin was unaltered in the presence of the AMPK inhibitor Compound C. By contrast, the inhibitory effect of AICAR on this metabolic index was fully prevented by Compound C, then establishing the AMPK-dependent action of this kinase activator. Finally, we observed that metformin also stimulated glucose utilization in primary cultures of mouse cortical neurons, whereas AICAR remained without effect in this cell type. As a whole, these results demonstrate that both metformin and AICAR strongly modify glucose metabolism in astrocytes and neurons. More specifically, metformin altered glucose metabolism in astrocytes by turning them into a more glycolytic state through an AMPK-independent mechanism which remains to be characterized.

Disclosures: I. Allaman: None. **G. Grenningloh:** None. **C. Barriere Borgioni:** None. **P.J. Magistretti:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.09/B34

Topic: B.11. Glial Mechanisms

Support: Office of Research and Sponsored Programs at Central Michigan University

College of Medicine at CMU

Field Neurosciences Institute

John G. Kulhavi Professorship

Title: *In vitro* reprogramming of adults rat astrocytes using SOX2

Authors: *S. T. PERUZZARO, S. M. RAUPP, M. M. ANDREWS, M. LU, Z. NAN, J. ROSSIGNOL, G. L. DUNBAR;
Central Michigan Univ., Mount Pleasant, MI

Abstract: Reprogramming astrocytes to neural stem cells and neurons is a potential strategy for neurological repair. Due to the proliferation of astrocytes and neuronal cell loss after ischemic stroke, reprogramming astrocytes to a neuronal phenotype may be a possible therapeutic strategy. Previous studies have shown that the sex-determining region, Y-box 2 (SOX2), is sufficient to induce mouse and human astrocytes to differentiate into neural stem cells and cells with a neuronal phenotype. This work aimed to re-program adult rat astrocytes to a neuronal phenotype, *in vitro*, using a SOX2 lentivirus. To our knowledge this is the first study looking at *in vitro* reprogramming of rat astrocytes. Furthermore, this study evaluated the survivability and effectiveness of reprogramming adult rat astrocytes with SOX2 under oxidative stress. This research has the potential to provide further information for the use of *in vivo* injections of SOX2 after ischemic injury.

Disclosures: S.T. Peruzzaro: Other; Field Neurosciences Institute Laboratory for Restorative Neurology, Program in Neuroscience. **S.M. Raupp:** Other; Program in Neuroscience. **M.M. Andrews:** Other; Field Neurosciences Institute Laboratory for Restorative Neurology, Program in Neuroscience, Department of Psychology. **M. Lu:** Other; Field Neurosciences Institute Laboratory for Restorative Neurology, Program in Neuroscience, Department of Psychology. **Z. Nan:** Other; Field Neurosciences Institute Laboratory for Restorative Neurology, Program in Neuroscience, Department of Psychology. **J. Rossignol:** Other; Field Neurosciences Institute Laboratory for Restorative Neurology, Program in Neuroscience, Department of Psychology, College of Medicine, Central Michigan University. **G.L. Dunbar:** Other; Field Neurosciences Institute Laboratory for Restorative Neurology, Program in Neuroscience, Department of Psychology,Field Neurosciences Inst., 4677 Towne Centre Rd. Suite 101 Saginaw, MI.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.10/B35

Topic: B.11. Glial Mechanisms

Support: PENED 0778

Title: Phenotypic changes during an endothelin-induced cell cycle in rat cortical astrocytes in culture

Authors: N. ZAFEIRAKOU[1], M. MELISSOURGOU[1], E. TSIRIMONAKI[1], A. GAITANAKI[2], N. SAKELLARIDIS[3], *D. A. MANGOURA[1];
[1]Biomed Res. Found Athens Acad., Athens, Greece; [2]Natl. and Kapodistrian Univ. of Athens, Athens, Greece; [3]Fac. of Medicine, Sch. of Hlth. Sciences, Univ. of Thessaly, Larissa, Greece

Abstract: Endothelin-1 (ET-1) is a mito¬gen for astro¬cytes and it is implicated in many CNS patho¬logies that involve reactive glio¬sis, through the activation of a com¬plex net¬work of in¬ter¬connected sig¬nal¬ing cascades that apparently include modification of phenotypic markers, like expression of GFAP. Indeed, other mitogens like prolactin concomitant signal proliferation and differentiation in conditions that fast gain in both numbers and extent of differentiation in the astrocytic population is required, for example during development or reaction to trauma. A major effector of both ET-1 receptors is ERK1/2, an important regu¬lator for both pro¬liferation and diffe¬rentiation. We have shown that among the receptor-proximal effects that lead to a biphasic ERK1/2 activation is an acute activation of Ras, which controls mainly the second amplification peak of ERK activation in rat cortical astrocytes in culture. We have now probed the longer term effects of ET-1 signalling in this model, namely phenotypic effects that take place within the first 24 hr of ET-1. First we verified that ET-1 signalling leads to proliferation, as the vast majority of cells had incorporated BrdU, and nuclear expression of CENPE and cyclin B were significantly upregulated, all pinpointing that astrocytes had progressed to G2. We then examined protein and mRNA levels of GFAP, S100b, and cJUN and found that by 6h there were small but significant upregulations, probably intended to increase the load of GFAP and S100b for cytokinesis. Once the cells progressed to S phase and de novo expression of cell cycle proteins started, transcription for GFAP and S100 was reduced. Next, using *in vivo* crosslinking of the proteins and high Triton-X-removal of membranes prior to fixation for best visualization of cytoskeleton, and confocal microscopy, we observed that the changes in GFAP expression with ET-1 were accompanied by a remarkable remodeling of GFAP positive filaments. Specifically, untreated cells exhibited thick, stubby, heavily loaded with GFAP processes, while cells exposed to ET-1 appeared with larger, enriched in GFAP somata and long, thin processes with fewer GFAP filaments. Importantly, when Ras activation was simulated with overexpression of v-Ras or siRNA-mediated depletion of the Ras-GAP neuro-fibromin (after nucleofections) we observed elongated processes that were highly enriched in GFAP; after 24h of ET-1, somata were further enlarged with very dense GFAP filaments. Collectively these results indicate that mitogen ET-1 activates concurrent signalling cascades that affect differentiation programs, controlled by Ras.

Disclosures: **N. Zafeirakou:** None. **M. Melissourgou:** None. **E. Tsirimonaki:** None. **A. Gaitanaki:** None. **N. Sakellaridis:** None. **D.A. Mangoura:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.11/B36

Topic: B.11. Glial Mechanisms

Support: International Promotion of Young Researchers "Montalcini Program" grant from the Italian Ministry of Education, University and Research

Title: Purines modulate growth of plasma membrane extensions in isolated astrocytes

Authors: *M. CHISARI, A. SCUDERI, M. A. SORTINO;
Univ. of Catania, Catania, Italy

Abstract: It has been shown that astrocytes exert their function in the tripartite synapse and make contact with brain vessels by plasma membrane (PM) extensions (perisynaptic processes and perivascular endfeet, respectively). Such PM extensions express aquaporin-4 (AQP-4) and K+ inward rectifying (Kir) channels, regulating both water and K+ homeostasis. Astrocyte activity is largely modulated by purines, such as adenosine. Therefore in the present study we focused on effects of purines in PM extension growth in astrocytes. In western blot analysis, we observed an increased expression of P2Y1 receptor over time in cultured hippocampal astrocytes. In isolated cells, we validated functional P2Y1 receptor (endogenous Gq protein coupled receptor) by monitoring calcium flux in living cells loaded with Fluo-4 calcium indicator, after stimulation with the selective agonist 2-methylthioadenosine diphosphate (2MeSADP, 100 μM). In order to evaluate PM extensions in astrocytes, living cells were stained with the fluorescent PM dye CellMask Orange, stimulated with 2MeSADP and imaged for 20 min. Number and length of PM extensions increased over time and such effect was prevented by pre-treatment with selective P2Y1 antagonist, MRS 2179 (1 μM). Since calcium mobilization by Gq-mediated pathway might contribute to PM extension growth, we performed experiments in presence of calcium chelator EGTA in the buffer. In cells loaded with Fluo-4 indicator and incubated with 10 μM EGTA for 10 min, astrocytes stimulated with 2MeSADP did not show either intracellular calcium increase or PM extension growth. In a different set of imaging experiments, cells treated with phospholipase C inhibitor U73122 (3 μM) showed a dramatic decrease of PM extensions in length and number. Based on this observation we hypothesized that reduction of PM extensions will affect both AQP-4 expression and Kir function. Western blot analysis of AQP-4, in conditions tested in imaging experiments, did not show any changes in protein level. Patch-clamp recordings were performed in isolated astrocytes applying voltage-steps (-180 mV to 60 mV) to elicit Kir current in basal condition and after U73122 application. A dramatic increase in current through K+ channels at negative potentials (-180 mV to -100 mV) was observed within 3 min in U73122. At positive potentials (20 mV to 60 mV), K+ current was reduced over time up to 10 min. These data show a dynamic modulation of astrocytes PM extensions by adenosine through P2Y1 receptors and the Gq-mediated pathway, suggesting their role in astrocyte buffering function, crucial for brain homeostasis.

Disclosures: **M. Chisari:** None. **A. Scuderi:** None. **M.A. Sortino:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.12/B37

Topic: B.11. Glial Mechanisms

Support: NINDS Grant RO1NS062784

Title: Functional maturation of astrocytic syncytium during postnatal development in mice hippocampus

Authors: S. ZHONG[1], C. M. KIYOSHI[1], B. MA[1], X. LIU[2], *M. ZHOU[1];
[1]Dept. of Neurosci., Ohio State Univ., Columbus, OH; [2]Dept. of Neurosci., Shanghai 10th People's Hospital, Tongji Univ. Sch. of Med., Shanghai, China

Abstract: It is generally believed that the basic function of astrocytes is achieved at the syncytial (system) level in the adult brain. However, the mechanism underlying this important functional aspect remains largely unknown. We show that, in the adult hippocampus, an extensive gap junction coupling synchronizes the membrane potential among coupled astrocytes to achieve a syncytial isopotential. However, when this occurs during the postnatal development and how this is associated with the maturation of the organizational patterning of syncytia are questions that remain to be answered. In the present study, the developmental change in syncytial organization was monitored by confocal morphometric analysis of hippocampal CA1 syncytia disclosed by intracellular biocytin loading through patch pipette. The functional maturation of the syncytial network in the same region was analyzed by use of K^+-free pipette solution. In the adult hippocampal CA1 region, a quasi-physiological membrane potential can be maintained due to a powerful electrical and ionic coupling provided by the associated syncytium. In contrast to the mature syncytium, K^+-free pipette solution can induce membrane potential depolarization. We further show that the syncytial function reaches to a mature level around P11. Altogether, we show that the syncytial function can be readily detected by our newly established method, and the functional maturation of hippocampal syncytium undergoes developmental regulation.

Disclosures: S. Zhong: None. **C.M. Kiyoshi:** None. **B. Ma:** None. **X. Liu:** None. **M. Zhou:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.13/B38

Topic: B.11. Glial Mechanisms

Support: Swedish Medical Research Council Grant 20116

Swedish Medical Research Council Grant 11548

EU FP 7 Program TargetBraIn (279017)

Swedish Brain Foundation

Swedish Stroke Foundation

Title: Complement peptide C3a promotes astrocyte survival in response to ischemic stress

Authors: *M. PEKNA, N. SHINJYO, Y. DE PABLO, M. PEKNY;
Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Astrocytes are the most numerous cells in the central nervous system with a range of homeostatic and regulatory functions. Under normal conditions as well as after ischemia, astrocytes promote neuronal survival. We have previously reported that the complement-derived peptide C3a stimulates neuronal differentiation of neural progenitor cells and protects the immature brain tissue against hypoxic-ischemic injury. Here, we studied the effects of C3a on the response of mouse cortical astrocytes to ischemia. We have found that chemical ischemia, induced by combined inhibition of oxidative phosphorylation and glycolysis, up-regulates the expression of C3a receptor in cultured primary astrocytes. C3a treatment protected wild-type but not C3a receptor deficient astrocytes from cell death induced by chemical ischemia or oxygen-glucose deprivation by reducing ERK signaling and caspase-3 activation. C3a attenuated ischemia-induced upregulation of glial fibrillary acidic protein, however the protective effects of C3a were not dependent on the presence of the astrocyte intermediate filament system. Pre-treatment of astrocytes with C3a during recovery abrogated the ischemia-induced neuroprotective phenotype of astrocytes. Jointly, these results provide the first evidence that the complement peptide C3a modulates the response of astrocytes to ischemia and increases their ability to cope with ischemic stress.

Disclosures: **M. Pekna:** None. **N. Shinjyo:** None. **Y. de Pablo:** None. **M. Pekny:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.14/B39

Topic: B.11. Glial Mechanisms

Support: Petro-Canada Young Innovator Award

MRU IRG

FST Seed Grant

NSERC-USRA

Title: The examination of the distribution of proteins within complex three-dimensional astrocytes

Authors: *A. L. BENEDIKTSSON[1], K. MILLOY[1], N. RASIAH[2], L. ALVIS[3];
[1]Biol., Mount Royal Univ., Calgary, AB, Canada; [2]Hotchkiss Brain Inst., [3]Fac. of Nursing, Univ. of Calgary, Calgary, AB, Canada

Abstract: Historically, the model of the synapse consisted of the pre- and post-synaptic neurons. New research suggests that astrocytes, a type of brain glial cell, play a crucial role in synapse structure and function. Astrocytes have a highly complex 3-D morphology that includes intricate connections with synapses, the vasculature and other astrocytes; as such, they have a variety of functions, including trophic and metabolic support of neurons, synaptic development and signalling, control of the microvasculature and co-ordination of neuronal networks. However, it is unclear whether astrocytes possess specialized regions to compartmentalize these functions. Our hypothesis is that astrocytes possess functional microdomains, specialized morphological compartments within a cell with a specific, highly localized array of proteins necessary for regional specialization and cellular function. To investigate this hypothesis we have combined DiOlistics, a biolistic technique for labelling the complete morphology of individual cells in brain tissue with lipophilic fluorescent dye, with immunocytochemistry to label individual proteins. We examined the distribution of these proteins within the complex three-dimensional morphology of individual adult murine brain astrocytes using confocal microscopy, and then determined their distribution in three-dimensions using Imaris™, a 3-D confocal rendering software. Our preliminary studies suggest differences between the distribution of proteins among astrocytes from similar brain regions, as well as astrocytes differing in GFAP expression.

Disclosures: **A.L. Benediktsson:** None. **K. Milloy:** None. **N. Rasiah:** None. **L. Alvis:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.15/B40

Topic: B.11. Glial Mechanisms

Support: NIH Grant RO1NS062784

NSF Grant DMS 1410935

NSF Grant DMS 0931642

Title: Modeling isopotentiality in the astrocytic syncytium

Authors: R. BUCKALEW, B. MA, M. ZHOU, *D. H. TERMAN;
Ohio State Univ., Columbus, OH

Abstract: Due to their high and selective membrane permeability to K+ ions, astrocytes act as nearly perfect potassium electrodes. As a result, experimental efforts to measure the strength and nature of the gap junction connections between astrocytes have been largely ineffective: in patch clamp experiments, the vast majority of injected current is shunted across the cell membrane rather than flowing through the gap junction channels. We have created a network model of the astrocytic syncytium to aid in theoretical understanding of the gap junction connections, as well as to verify recent experimental results from Ma et al using a new cytosol-replacement protocol that avoids the problem of current shunting. Each astrocyte is modeled as a single compartment following the Hodgkin-Huxley formalism. The model includes variables for membrane potential, K+ and Na+ concentrations (intra- and extra-cellular), as well as bath and electrode pipette concentrations. Electrical currents and ionic fluxes across the cell membrane and through gap junction channels are calculated according to the Goldman-Hodgkin-Katz equation for electrodiffusion, using ionic permeabilities obtained from the literature. The model predicts the recently discovered ‘isopotential’ effect in coupled astrocytes, whereby membership in a syncytium serves to synchronize membrane potentials among the cells and prevent large depolarizations due to localized ionic or electrical perturbations. The model is further able to predict the strength of coupling across the gap junction channel via a fitting parameter s=P_(K,gap)/P_K . The predicted value of s is 1, corresponding to a maximum conductance of 240.5 nS across a gap junction connection. The major physiological implication of the isopotential effect is increased capacity for K+ uptake from the extracellular space, which greatly enhances the effectiveness of K+ spatial buffering by astrocytes. Our model predicts that a syncytium consisting of cells coupled with 11 neighbors is sufficient to maintain a physiological membrane potential in the presence of a 20 mM extracellular increase in K+ concentration, and to efficiently distribute the excess potassium across the syncytium on a time scale of seconds.

Disclosures: R. Buckalew: None. **B. Ma:** None. **M. Zhou:** None. **D.H. Terman:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.16/B41

Topic: B.11. Glial Mechanisms

Support: RO1NS062784

DMS 1410935

DMS 0931642

Title: Gap junction coupling confers isopotentiality on astrocyte syncytium

Authors: *B. MA[1], R. BUCKALEW[2], Y. DU[1], C. KIYOSHI[1], C. ALFORD[1], W. WANG[1], D. MCTIGUE[1], J. ENYEART[1], D. TERMAN[3], M. ZHOU[1];
[1]Dept. of Neurosci., [2]Mathematical Biosci. Inst., [3]Dept. of Mathematics, The Ohio State Univ., Columbus, OH

Abstract: Astrocytes are extensively coupled through gap junctions into a syncytium. However, the basic role of this major brain network remains largely unknown. Here, we use electrophysiological and computational modeling methods to demonstrate that the membrane potential (VM) of an individual astrocyte in a hippocampal syncytium, but not in a single, freshly isolated cell preparation, can be well-maintained at quasi-physiological levels when recorded with reduced or K+ free pipette solutions that alter the K+ equilibrium potential to non-physiological voltages. We show that an astrocyte's associated syncytium provides powerful electrical and ionic coupling that synchronizes the astrocyte's VM to levels comparable to its neighbors. Functionally, this minimizes VM depolarizations due to elevated levels of local extracellular K+ and thereby maintains a sustained driving force for highly efficient K+ uptake. Thus, gap junction coupling functions to achieve an isopotentiality in astrocytic networks, whereby a constant extracellular environment can be powerfully maintained for crucial functions of neural circuits

Disclosures: B. Ma: None. **R. Buckalew:** None. **Y. Du:** None. **C. Kiyoshi:** None. **C. Alford:** None. **W. Wang:** None. **D. McTigue:** None. **J. Enyeart:** None. **D. Terman:** None. **M. Zhou:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.17/B42

Topic: B.11. Glial Mechanisms

Support: NE DHHS Stem Cell 2012-05

Alzheimer's Association NIRP-12-258440

Title: Loss of GSK-3 causes abnormal astrogenesis and behavior in mice

Authors: E.-M. JUNG, *W.-Y. KIM;
Developmental Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Altered activity of glycogen synthase kinase-3 (GSK-3) is associated with psychiatric diseases and neurodegenerative diseases. GSK-3 is a key regulator in multiple aspects of neuronal differentiation in the brain. However, little is known about the role of GSK-3 in astrocyte regulation. To examine the role of GSK-3 in astrocytes, we generated a conditional knockout mouse using a GFAP-cre driver, in which the GSK-3 alpha and beta genes are deleted in astrocytes. We found that GFAP-cre-mediated GSK-3 deletion led to a larger brain. The number and size of astrocytes were increased in GSK-3 mutant brains. The levels of GFAP and phospho-STAT3, indicators of astrogenesis, were elevated in GSK-3 mutants. Furthermore, we found upregulation of astrocyte regulatory molecules such as phospho-AKT, phospho-S6, and cyclin D in GSK-3 mutant brains. Finally, GSK-3 mutant mice exhibited aberrant anxiety and social behavior. Our results suggest that GSK-3 plays a significant role in astrocyte regulation and behavioral control in mice.

Disclosures: E. Jung: None. **W. Kim:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.18/B43

Topic: B.11. Glial Mechanisms

Support: CIHR doctoral student award

Title: Nitric oxide modulation of phosphodiesterase activity and cAMP levels in astrocytes

Authors: *R. KO, H. B. CHOI, B. A. MACVICAR;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Astrocytic cAMP signaling plays an important role in several crucial physiological processes in the brain, including metabolic coupling to neurons. However, the regulation of cAMP levels by phosphodiesterases in astrocytes has not been determined. Here we investigate the hypothesis that phosphodiesterase 3 (PDE3) is an important regulator of astrocytic cAMP levels in the brain. The presence of PDE3 in astrocytes could have intriguing functional impact as this PDE is inhibited by cGMP. Recent transcriptome and protein analyses have confirmed high levels of astrocytic PDE3. Interestingly, nitric oxide (NO)-stimulated cGMP production has been demonstrated to potentiate cAMP levels via PDE3 inhibition in blood vessels. To examine the role of PDE3 in modifying cAMP levels, we performed ELISAs in both acute rat hippocampal brain slices and primary astrocyte cultures. Our preliminary data indicate that the PDE3 inhibitor, cilostamide (10μM), enhances forskolin-induced cAMP increase. In addition,

application of the nitric oxide (NO) donor, SNAP (100 μM), also potentiated the cAMP increase, consistent with PDE3 sensitivity to cGMP. To further confirm that NO-induced cGMP is modulating cAMP levels through PDE3 inhibition, the experiment will be repeated in the presence of the soluble guanylyl cyclase inhibitor, ODQ. We are also examining the downstream consequences of increasing astrocytic cAMP by NO modulation. We have previously shown that activation of metabotropic glutamate receptors on astrocytes leads to prostaglandin E2 release and subsequent vasodilation. Intriguingly, cAMP elevation leads to lactate efflux from astrocytes which inhibits prostaglandin reuptake transporters. Hence NO may potentiate vasodilation by enhancing PGE2 signaling. Additionally, cAMP also modulates connexin43 (Cx43) expression and gap junction assembly in hepatoma cells. We therefore predict that NO can alter Cx43 levels and subsequent gap junction coupling of astrocytes. Thus we will perform immunoblotting and dye flux imaging to assess the level of plasma membrane Cx43 and astrocyte coupling, respectively. NO can be generated by either constitutive or inducible NO synthase in several different cell types such as interneurons and microglia. This raises an intriguing possibility that crosstalk may occur between different cell types and astrocytes to modulate cAMP-dependent pathways in astrocytes that are important in maintaining the healthy brain.

Disclosures: **R. Ko:** None. **H.B. Choi:** None. **B.A. MacVicar:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.19/B44

Topic: B.11. Glial Mechanisms

Support: NINDS Grant RO1NS062784

Title: Astrocytic syncytial isopotentiality in grey matter and white matter of adult mice

Authors: ***S. ZHONG**[1], C. M. KIYOSHI[1], B. MA[1], X. LIU[2], M. ZHOU[1];
[1]Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH; [2]Dept. of Neurol., Shanghai 10th People's Hospital, Tongji Univ. Sch. of Med., Shanghai, China

Abstract: Within an astrocytic syncytium of adult mice hippocampus, a powerful electrical and ionic coupling equalizes the membrane potentials among the coupled cells to achieve a syncytial isopotentiality. The existence of a syncytial isopotential is indicated by the ability of syncytial coupling to maintain a physiological membrane potential for any individual astrocyte in the network when K^+ ions were totally substituted by other monovalent cations in the whole-cell recording pipette solution, whereas the same experimental condition depolarizes freshly dissociated single astrocyte to ~0 mV as predicted by the Goldman-Hodgkin-Katz equation. To answer whether the observed syncytial isopotential in the adult hippocampus represents a general

functional aspect of astrocyte network, the same experimental method was used to examine astrocyte syncytia in other grey matter regions, such as hippocampal dentate gyrus and various subregions of cerebral cortex, as well as the white matter of the corpus callosum. In confocal morphometric analysis, we show that the density and spatial organization patterns do vary within various grey matters, and between grey and white matter. However, independent of these variations, a similar syncytial isopotentiality always exists in all the brain regions examined. Therefore, syncytial isopotential is most likely a universal characteristic of the astrocyte syncytial network that serves as a fundamental mechanism for the homeostatic support function of astrocytes throughout the brain.

Disclosures: **S. Zhong:** None. **C.M. Kiyoshi:** None. **B. Ma:** None. **X. Liu:** None. **M. Zhou:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.20/B45

Topic: B.11. Glial Mechanisms

Support: Marie Curie ITN Neurokine

Title: Highly purified adult astrocytes display diverse stimulus-dependent activation and cytokine secretion

Authors: H. ZHANG[1], M. JUNGBLUT[1], S. REISS[1], L. ZATRIEB[1], S. RÜBERG[1], L. WILLNOW[1], S. WILD[1], J. KOLLET[1], S. TOMIUK[1], R. FEKETE[2], *A. BOSIO[1,2];
[1]Miltenyi Biotec, Bergisch Gladbach, Germany; [2]Fluidigm Corp., South San Francisco, CA

Abstract: The inflammatory response in the central nervous system is a multifactorial process involving resident neural cells as well as infiltrating immune cells. These cells communicate by secreting cytokines, chemokines, and growth factors. Astrocytes are known to play multiple roles in neuroinflammation but there are no detailed studies to classify the heterogeneity of activated astrocytes according to their cytokine and chemokine secretion profile. In the past we have set up an automated dissociation procedure for neonatal brain using the gentleMACS Octo Dissociator with an optimized enzymatic treatment. For isolation and culture of adult neural cells we have further improved the method and included a novel protocol for removal of debris and erythrocytes, yielding 2-4 x10E6 living cells per mouse brain. To assess and compare neonatal and adult astrocyte diversity we first analyzed their transcriptome by mRNA sequencing of single cells. Astrocytes were separated to a purity of > 98,5 % using MicroBeads (MACS®) coupled to a specific pan-astrocyte marker, Anti-ACSA-2. The C1 Single-Cell Auto Prep System (Fluidigm) was used for capturing single cells in integrated fluidics circuits (IFCs), and

subsequent reverse transcription and cDNA amplification in a nanoliter reaction volume. The single cell transcriptome analyses revealed a divers molecular profile of both neonatal and adult astrocytes. Next, we characterized the cytokine secretion profile of activated neonatal and adult astrocytes. Immunocytochemistry analyses showed that neonatal and adult astrocytes expressed a broad range of cytokines and chemokines. When lipopolysaccharides (LPS) or conditioned media from LPS-stimulated microglia were added to neonatal astrocytes, an increased level of GM-CSF was detected by MACSPlex Cytokine Kit (Miltenyi Biotec). In case of adult astrocytes a distinct secretion profile dominated either by GM-CSF or IL23 was detected when activating adult astrocytes with LPS+TNFa or LPS+IFNg, respectively. This points to diverse astrocyte subtypes which are capable of reacting to different stimuli. In summary, we present a novel technology to purify functional astrocytes from neonatal and adult mouse brain paving the way for sophisticated molecular and cellular analyses. Single cell transcriptome analyses revealed general molecular diversity of astrocytes while cytokine secretion analyses of activated astrocytes demonstrate their functional diverse and stimulus-dependent polarization. The characterization of astrocyte polarization will help to further understand and modulate the inflammatory response in the CNS in diseases such as multiple sclerosis.

Disclosures: **H. Zhang:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH. **M. Jungblut:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH. **S. Reiss:** A. Employment/Salary (full or part-time):; Miltenyi Biotec.GmbH. **L. Zatrieb:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH. **S. Rüberg:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH. **L. Willnow:** A. Employment/Salary (full or part-time):; Miltenyi Biotec. **S. Wild:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH. **J. Kollet:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH. **S. Tomiuk:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH. **R. Fekete:** A. Employment/Salary (full or part-time):; Fluidigm Corporation. **A. Bosio:** A. Employment/Salary (full or part-time):; Miltenyi Biotec GmbH.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.21/B46

Topic: B.11. Glial Mechanisms

Support: the research fund of the IRP, NIMH, NIH

Title: Valproic acid promotes fibroblast growth factor 21 gene expression and neurite-like elongation through inhibition of HDAC2 and 3 in glial cells

Authors: ***Y. LENG**, J. WANG, Z. WANG, H.-M. LIAO, P. LEEDS, D.-M. CHUANG; Mol. Neurobiol Section, Natl. Inst. Mental Health/NIH, Bethesda, MD

Abstract: Fibroblast growth factor 21 (FGF-21) is an identified regulator of glucose and lipid metabolism, and has been proposed as a potential target for treating diabetes, obesity, and hyperlipidemia. Valproic acid (VPA), a mood stabilizer and an anticonvulsant drug, is a histone deacetylase (HDAC) inhibitor, and regulates gene expression via chromatin remodeling through epigenetic mechanisms. Recently, our lab reported that FGF-21 mRNA and protein can be markedly induced in rat brain-derived neurons by combined treatment with the mood stabilizers VPA and lithium, while VPA alone was ineffective (Leng et al., Mol Psychiatry 2015). As an extension of our previous work, this study investigated the effects of VPA and other HDAC inhibitors on the expression of FGF-21 in glial cells. Incubation of C6 glioma cells with VPA and other pan HDAC inhibitors such as sodium butyrate, trichostatin A (TSA) and SAHA, all robustly increased FGF-21 mRNA levels in a dose and time-dependent manner. Class I HDAC inhibitors such as MS-275, apicidin, FK-228 and MGCD103 also caused a dose-dependent increase in FGF-21 mRNA; however, the HDAC6-specific inhibitors rocilinostat (ACY-1215) and tubastatin A had no significant effects. To identify the class I HDAC isoform(s) involved in the regulation of FGF-21 expression, we employed lentiviral shRNA-mediated knockdown technology. Knock down of HDAC2 or 3 isoforms in C6 glioma with their specific shRNA, elevated FGF-21 mRNA level by 8- or 3-fold, respectively. In contrast, HDAC1 isoform knockdown produced no FGF-21 mRNA expression changes in these cells. Knock-down of either HDAC2 or 3, but not HDAC1 markedly increased neurite-like elongation in C6 cells, similar to effects of VPA. Furthermore, knock-down of endogenous FGF-21 with lentivirus-mediated shRNA significantly reduced VPA-induced neurite-like elongation in C6 cells. Conversely, lentivirus-mediated overexpression of recombinant FGF-21 increased the length of neurite projection. Additionally, in rat cortical primary astroglia-enriched cells, VPA and SAHA upregulated the FGF-21 mRNA level by 4- and 8-fold, respectively, and this was associated with a marked increase in neurite-like projections in cortical astrocytes. Taken together, our results suggest that inhibition of HDAC2 and 3 by VPA or other HDAC inhibitors up-regulates FGF-21 and neurite-like elongation in glial cells. The neurobiological significance of FGF-21 up-regulation in glia requires further investigation.

Disclosures: **Y. Leng:** None. **J. Wang:** None. **Z. Wang:** None. **H. Liao:** None. **P. Leeds:** None. **D. Chuang:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.22/B47

Topic: B.11. Glial Mechanisms

Title: Monoacylglycerol lipase inhibitors block astrocyte cytokine/chemokine secretions following LPS *in vitro*

Authors: *S. SUTTON[1], Y. HE[2], N. TAYLOR[2], N. DERECKI[2], A. BHATTACHARYA[2], P. BONAVENTURE[2];
[1]Neurosci. Drug Discovery, Janssen Res. & Develop., San Diego, CA; [2]Neurosci. Drug Discovery, Janssen Res. & Development, LLC, San Diego, CA

Abstract: Recent publications have shown stimulation of brain cytokines, chemokines and prostaglandin E2 (PGE2) in mice following peripheral injections of 20 mg/kg lipopolysaccharide (LPS). In these studies monoacylglycerol lipase (MGL) inhibitors have been shown to reduce LPS-stimulated cytokine and PGE2 elevations in brain extracts. These observations suggest MGL inhibitors may be capable of reducing neuroinflammation. Here we use cultured primary mouse astrocytes to model neuroimmune effects of MGL inhibitors, comparing those effects to FAAH inhibitor JNJ-40355003. Results of these studies show secretion of cytokines such as Il-6, Il-13, IP-10/CXCL-10, KC/CXCL-1, MCP-1 and RANTES are stimulated by 10 ng/ml LPS in primary mouse astrocytes, and these secretions are blocked by 10 uM of the MGL inhibitors but not by 10 uM of the FAAH inhibitor. While a wide variety of cells participate in neuroimmune signaling including astrocytes, microglia, endothelial cells, pericytes, neurons and infiltrating peripheral immune cells, these observations suggest aspects of brain cytokine modulation by MGL inhibitors observed *in vivo* can be modeled with primary astrocytes *in vitro*. References: JE Schlosburg et al, Nature Neuroscience. 13, 1113-1121 (2010) DK Nomura et al, Science. 334, 809-813 (2011)

Disclosures: **S. Sutton:** A. Employment/Salary (full or part-time):; Janssen Research & Development, LLC. **Y. He:** A. Employment/Salary (full or part-time):; Janssen Research & Development, LLC. **N. Taylor:** A. Employment/Salary (full or part-time):; Janssen Research & Development, LLC. **N. Derecki:** A. Employment/Salary (full or part-time):; Janssen Research & Development, LLC. **A. Bhattacharya:** A. Employment/Salary (full or part-time):; Janssen Research & Development, LLC. **P. Bonaventure:** A. Employment/Salary (full or part-time):; Janssen Research & Development, LLC.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.23/B48

Topic: B.11. Glial Mechanisms

Support: NIH RO1 045926

National Science Foundation Graduate Research Fellowship under Grant No. 2012142088

Title: Untangling GFAP via Alexander disease patient derived astrocytes

Authors: *J. JONES[1,2], R. KRENCIK[2], M. DOERS[1], T. HAGEMANN[1], R. BRADLEY[1], M. DUBOVIS[1], A. MESSING[1], S.-C. ZHANG[1];
[1]Univ. of Wisconsin-Madison Wasiman Ctr., Madison, WI; [2]Ophthalmology, Univ. of California San Francisco, San Francisco, CA

Abstract: Glial fibrillary acidic protein (GFAP) is widely regarded as an astrocyte marker. However, its function in astrocytes remains largely unknown. Point mutations in either GFAP allele are sufficient to induce a disease known as Alexander disease. Though rare, the existence of Alexander disease suggests critical roles for GFAP. We endeavored to investigate Alexander disease in an effort to not only understand disease mechanisms, but also to explore the function of GFAP within astrocytes. To that end, we established induced pluripotent stem cells (iPSCs) from Alexander disease patients with different GFAP mutations. We also generated isogenic iPSCs by correcting the GFAP mutations with CRISPR/CAS9. Strikingly, astrocytes differentiated from patients' iPSCs exhibited GFAP aggregation, hallmark pathology of Alexander disease. Functional analysis revealed altered metabolic properties,glutamate buffering capacity, and interaction with neurons. These results highlight the functions of GFAP beyond a cytoskeletonprotein. This study has also demonstrated the feasibility of human disease modeling *in vitro* as well as elucidated potential future directions for understanding roles of GFAP and astrocytes in human health and disease.

Disclosures: J. Jones: None. **R. Krencik:** None. **M. Doers:** None. **T. Hagemann:** None. **R. Bradley:** None. **M. Dubovis:** None. **A. Messing:** None. **S. Zhang:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.24/B49

Topic: B.11. Glial Mechanisms

Support: NINDS Grant NS059005

NIA Grant AG032349

ISF

Title: Astrocytes engulf apoptotic cells through the Megf10 scavenger receptor

Authors: *T. IRAM[1,2], D. FRENKEL[1,3], J. EL KHOURY[4];
[1]Sagol Sch. for Neuroscience, Tel Aviv Univ., Tel Aviv-Yafo, Israel; [2]Dept. of Neurobilology, Tal Aviv Univ., TelAviv, Israel; [3]Dept. of Neurobilology, Tal Aviv Univ., Tel Aviv, Israel; [4]Ctr. for Immunol. and Inflammatory Dis., Mass Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: Efficient phagocytic clearance of apoptotic cells in the central nervous system (CNS) is necessary to avoid toxicity and auto-immunity. Astrocytes, the most abundant cells in the brain, were shown to take part in debris clearance and tissue repair in several brain pathologies such as Mutliple Sclerosis, Alzheimer's disease and traumatic brain injury. Extensive work has been done on the clearance of apoptotic cells in *C. elegans* and *Drosophila* and several receptors such as cell death abnormality protein 1(CED-1) and Draper have been identified. However, in the mammalian and rodent CNS, the receptors involved in the clearance of apoptotic cells have not been definitively identified yet. Multiple EGF-like domains 10 (Megf10) is a type-I receptor that consists of all the structural features of CED-1, positioning it as a high-potential candidate for the mammalian CED-1 homologue. We and others have found that Megf10 is highly enriched in the brain, specifically in astrocytes. Moreover, Megf10 mediates engulfment of apoptotic neurons in-vitro. Furthermore, to study the function of Megf10 as an engulfment receptor in-vivo we generated a Megf10 knockout (Megf10-KO) mouse and found that lack of one or two copies of Megf10 results in an accumulation of apoptotic cells in the developing cerebellum and to locomotor deficits in the adult mouse. Notably, our results demonstrate for the first time, a role for Megf10 expressed on astrocytes in apoptotic-cells clearance ex-vivo, indicating that the accumulation of apoptotic cells in-vivo might be due to altered astrocytic-phagocytic activity. This data is highly relevant to EMARDD patients, which on top of the obvious muscle deficits might have cerebellum-related motor impairments due to failure of proper clearance of apoptotic cells. All together, these findings indicate that Megf10 plays an important role in astrocyte-mediated clearance of apoptotic cells.

Disclosures: **T. Iram:** None. **D. Frenkel:** None. **J. El Khoury:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.25/B50

Topic: B.11. Glial Mechanisms

Title: Mitochondrial glutamate carrier SLC25A22 inhibition in astrocytes does not result in energy failure

Authors: ***F. MOLINARI**[1], E. GOUBERT[1], Y. MIRCHEVA[1], F. M. LASORSA[2], J. C. SUTERA-SARDO[1], H. BECQ[1], F. PALMIERI[2], L. PALMIERI[2], L. ANIKSZTEJN[1]; [1]INMED - INSERM U901, Marseille, France; [2]Dept. of Pharmaco-Biology, Lab. of Biochem. and Mol. Biol., Bari, Italy

Abstract: The SLC25 carrier family drives the import of a large diversity of metabolites into mitochondria, a key cellular structure involved in many metabolic functions such as ATP synthesis by oxidative phosphorylation, tricarboxylic acid cycle or fatty acid oxidation.

Dysfunctions of these carriers result in severe diseases affecting organs requiring a high energy production such as muscles, heart, liver and brain. Moreover, direct dysfunction of the mitochondrial respiratory chain resulting in ATP synthesis deficiency are involved in diverse epileptic syndromes such as MERRF, MELAS, Alpers syndrome and some cases of early epileptic encephalopathies. In this study, we focus on the mitochondrial glutamate carrier SLC25A22 (also named GC1), which is involved in early epileptic encephalopathy and migrating partial seizures in infancy, and wondered if these syndromes could be due to a defect in ATP synthesis. In brain, GC1 is highly expressed in astrocytes and is the principal glutamate gate to entry into mitochondria. Here, we combined RNA interference to trigger GC1 knockdown to electroporation-mediated transfection using the Neon® Transfection System in order to target a high number of primary astrocytes. We measured several biochemical parameters such as NADH, mitochondrial membrane potential and level of cytosolic ATP. We found that the global ATP amount was similar in presence or absence of GC1. Our results suggest that GC1 is not crucial for energy formation and that GC1-related epileptic encephalopathies are not due to an energy failure in astrocytes.

Disclosures: **F. Molinari:** None. **E. Goubert:** None. **Y. Mircheva:** None. **F.M. Lasorsa:** None. **J.C. Sutera-Sardo:** None. **H. Becq:** None. **F. Palmieri:** None. **L. Palmieri:** None. **L. Anikszteijn:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.26/B51

Topic: B.11. Glial Mechanisms

Support: Ministerio de Economía Grant BFU2011-30456

Comunidad de Madrid Grant S2010/BMD-2402 MITOLAB-CM

Collaborative Joint Project awarded by IRDiRC and funded by ISCIII grant IR11/TREAT-CMT

institutional grant from the Fundación Ramón Areces to the Centro de Biología Molecular Severo Ochoa

I.J. is recipient of Programa Predoctoral de Formación de Personal Investigador No Doctor from the Departamento de Educación, Política Lingüística y Cultura del Gobierno Vasco

I.L.F is recipient of a contract from Comunidad de Madrid Grant S2010/BMD-2402 MITOLAB-CM

Title: Regulation of mitochondrial respiration in astrocytes

Authors: I. JUARISTI[1,2,3], A. DEL ARCO[1,2,3,4], *J. A. ESTEBAN[5], J. SATRUSTEGUI[1,2,3], I. LLORENTE-FOLCH[1,2,3];
[1]Dept. de Biología Molecular, Ctr. de Biología Mol. Severo Ochoa, Consejo Superior de Dept. de Investigaciones Científicas-Universidad Autónoma de Madrid (CSIC-UAM), Madrid, Spain; [2]Ctr. de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain; [3]Inst. de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Madrid, Spain; [4]Ctr. regional de Investigaciones Biomédicas, Facultad de Ciencias Ambientales y Bioquímica, Univ. de Castilla La Mancha, Toledo, Spain; [5]Ctr. De Biologia Mol. Severo Ochoa, Madrid, Spain

Abstract: In the brain, calcium regulates mitochondrial respiration both by activation of ATP consumption and by Ca signaling. Ca signaling in mitochondria may regulate respiration: i) after Ca entering through mitochondrial uniporter (MCU), activating mitochondrial dehydrogenases and F0F1-ATPsynthase; or ii) activating Ca-binding mitochondrial carriers, ARALAR and SCaMCs from the outer face of the inner mitochondrial membrane, which involves metabolite supply. In neurons, ARALAR contributes significantly to workload and Ca-induced respiration (1). In brain astrocytes, ARALAR levels are undetectable and this work aims to study the metabolic response of cultured astrocytes to different workloads and the possible role of Ca in response, using different ligands, glutamate (L-Glu) and ATP. L-Glu is transported into the astrocytes resulting in increased Na+ uptake, which drives ATP breakdown, and also acts on metabotropic L-Glu receptors. L-Glu (200 μM - 500 μM) produced a small rise in Cai in cultured astrocytes, increased glucose utilization, lactate production and stimulated oxygen consumption in an acute and stable way (OCR, by Seahorse XF24 Extracellular Flux Analyzer) and maximal uncoupled respiration (MUR) in the presence of 2.5 mM glucose. A transportable but non-metabolizable glutamate analog, D-aspartate (D-Asp) (500 μM), which also increases Cai, stimulated respiration in a way similar to L-Glu, but failed to increase MUR, clearly showing that L-Glu is used as respiratory substrate by cultured astrocytes. L-Glu and D-Asp-stimulation of respiration was only slightly reduced in the absence of Cao, consistent with a major role of ATP demand in stimulation of OCR. ATP (100 μM - 1 mM) activates metabotropic and ionotropic purinergic receptors and induces a larger Cai rise than L-Glu in cultured astrocytes. ATP caused an increase in glucose utilization and lactate production, and also an acute and transient rise in OCR, which was dependent on Cao. Whether this is due to increased Ca-dependent workload or to the effects of Ca on upregulation of respiration is part of the ongoing work. Remarkably, MUR was also Ca-dependent, especially in astrocytes exposed to high ATP concentrations. It is likely that glycogen breakdown via Ca-dependent adenylate cyclase and PKA (2) plays a role in increasing MUR in the presence of Ca. Further studies are required to dissect the role of MCU and SCaMC-3 and Ca-dependency with each stimulus. 1. Llorente-Folch et al. J Neurosci (2013) 33:13957-71. 2. Müller MS et al. Glia (2014) 62:154-62

Disclosures: I. Juaristi: None. **A. del Arco:** None. **J.A. Esteban:** None. **J. Satrustegui:** None. **I. Llorente-Folch:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.27/B52

Topic: B.11. Glial Mechanisms

Support: RO1NS077773

P30HD026979

T32-GM008076

F31-NS086255

Title: Astrocytic mitochondria undergo delayed fragmentation and degradation in response to an *in vitro* model of ischemia/reperfusion injury

Authors: *J. C. O'DONNELL[1], J. G. JACKSON[2], M. B. ROBINSON[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Several recent studies show that mitochondria are found in astrocytic processes and may even be found in the fine peripheral astrocytic process (PAPs). In fact, we recently showed that these mitochondria are mobile and that neuronal activity positions mitochondria near glutamate transporters and excitatory synapses (Jackson et al., J. Neuroscience, 2014). Mitochondrial dysfunction in neurons and excitotoxicity from failure of astrocytic glutamate uptake are at the core of delayed neuronal death after a transient ischemic insult. Aside from inducing hypothermia, this secondary pathology is currently untreatable. We hypothesized that astrocytic mitochondrial dynamics would be altered during secondary pathology; this could impair glutamate clearance and exacerbate excitotoxicity. To test this hypothesis we exposed organotypic hippocampal slice cultures to transient (30 min) oxygen glucose deprivation (OGD) after selective biolistic transfection of astrocytes to label mitochondria and plasma membrane. In agreement with the literature and as is observed with stroke *in vivo*, we observe preferential and delayed cell death in area CA1 that was blocked by ionotropic glutamate receptor (iGluR) antagonists. We found that this insult results in a dramatic, delayed loss of mitochondria in astrocytic processes. Mitochondrial fractionation (increased number, decreased length) was apparent by 9 hrs after OGD injury, followed by progressive loss of mitochondria from 12 to 24 hrs. By 18 hrs after injury, the fraction of process length occupied by mitochondria was reduced by ~40%, and mitochondrial length was reduced by ~60%. We observed a time-dependent increase in the colocalization of mitochondria with a transfectable autophagosome marker (EGFP-LC3B) indicative of increased mitophagy. Treatment with N-methyl-D-aspartate (100 μM for 30 min), which causes acute neuronal death, also caused a loss of astrocytic mitochondria. iGluR antagonists partially blocked the effects of OGD on astrocytic mitochondria. Cyclosporin-A, which inhibits mitochondrial fission and increases mitochondrial capacity for calcium buffering, also attenuated the loss of mitochondria after OGD and was protective. We are currently investigating possible physiologic and pathologic consequences of this loss of mitochondria. Understanding the mechanisms underlying this loss of astrocytic

mitochondria after transient OGD could provide new therapeutic targets for the currently untreatable secondary pathology following transient ischemic stroke.

Disclosures: **J.C. O'Donnell:** None. **J.G. Jackson:** None. **M.B. Robinson:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.28/B53

Topic: B.11. Glial Mechanisms

Support: NIH Grant NS R01084817-01

NIH Grant DA R01033966-01A1

Title: Acute exposure to methamphetamine decreases activity of K2P channels and voltage-gated K+ channels in primary human fetal astrocytes

Authors: ***S. DAVE**, C. YU, C. E. KHODR, M. SEATON, L. CHEN, L. AL-HARTHI, X.-T. HU;
Rush Univ. Med. Ctr., Chicago, IL

Abstract: Methamphetamine (Meth) is a potent and commonly-abused psychostimulant. Chronic exposure to Meth induces decreased neuronal activity in the medial prefrontal cortex and nucleus accumbens (key regulators of cognition and addiction in the reward pathway), which may contribute to the mechanisms underlying Meth addiction. It is not fully understood whether such decrease results from alterations in synaptic/intrinsic excitability of neurons, or dysregulation of the extracellular environment (e.g., glutamate and K^+ levels) mediated by surrounding astrocytes. To fill this knowledge gap, we assessed the acute effects of Meth on functional activity of certain voltage-gated ion channels in the cell membrane of primary human fetal astrocyte (HFA) using whole-cell voltage-clamp recording. We firstcharacterized the activity of K^+ channels in HFAs. Under blockade of voltage-gated Na^+ and Ca^{2+} channels, we found that HFAs showed a resting membrane potential (RMP) of -48±2 mV (-26 to -73 mV range). Blockade of the two-pore domain K^+ channel (K_{2P}) with quinidine (1 mM) induced depolarization of RMP in vehicle-treated control cells ($p<0.05$), revealing the role of K_{2P} channels in maintaining RMP of HFAs. HFAs showed predominant voltage-sensitive, out-flowing K^+ currents (VGKCs, a characteristic of immature or reactive astrocytes) at depolarized membrane potential levels (V_m, 60-100 mV). Bath-applied TEA (a selective K^+channel blocker, 20 mM) suppressed these currents ($p<0.001$), indicating that these currents were mediated by, and passed through activated VGKC channels. In another set of experiments, voltage-gated Ca^{2+} currents were not found in HFAs. Exposure of HFAs to acute Meth (20, 100, or 300 μM for 3-6 hrs) induced depolarization of RMP (all $p<0.05$). With K_{2P} channel blockade, Meth-induced

depolarization showed no significant difference *vs*. that induced by vehicle (p=0.5), suggesting that Meth decreased K_{2P} channel activity. Exposure to Meth (20, 100, or 300 μM) also induced a significant reduction in VGKCs compared to vehicle-treated controls (all p<0.05). There was no significant difference between the reduced VGKCs induced by TEA and by TEA+Meth (p>0.2), indicating that acute Meth also induced a decrease in VGKC channel activity. Together, these novel findings suggest that Meth disturbs HFA activity partly by decreasing activity of K_{2P} and VGKC channels. This could reduce K^+ out-flow and consequentially decrease local extracellular K^+ levels ($[K^+]_e$). If a similar decrease of $[K^+]_e$ occurs in mature astrocytes in the brain, it may contribute to lowering $[K^+]_e$, and thereby decreasing excitability of neurons surrounded by astrocytes.

Disclosures: S. Dave: None. **C. Yu:** None. **C.E. Khodr:** None. **M. Seaton:** None. **L. Chen:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.29/B54

Topic: B.11. Glial Mechanisms

Support: HHMI PreDoc (RVR)

R01EY007023

U01NS090473

EF1451125

Simons Foundation (MS)

Title: Robust and reliable Ca^{2+} response in microdomains of astrocytes

Authors: *R. GARCIA, R. RIKHYE, M. SUR;
Brain & Cognitive Sci., MIT, Cambridge, MA

Abstract: Astrocytic intracellular Ca^{2+} signaling has come to light as a prominent feature of neuronal-glial interactions. The majority of astrocyte Ca^{2+} signaling studies are performed in either culture or *in situ* brain slices, both of which rely on electrical stimulation or pharmacological methods to examine the spatial and temporal coding of astrocyte Ca^{2+} signals. We have investigated visually evoked Ca^{2+} responses in astrocytes of the visual cortex of awake, head-fixed mice using two-photon microscopy. Initially, our studies involved the use of viral-mediated delivery of genetically encoded calcium indicators. However, in order maintain an intact and unperturbed cortex, we have chosen to use a recently developed line of conditional

transgenic animals that express GCaMP5G in astrocytes throughout the mouse brain. We have found that Ca^{2+} transients in distal processes of cortical astrocytes are more frequent than those observed in anesthetized preparations, exhibiting a variable relationship to somatic responses. Furthermore, we found discrete structural regions of distal processes of single astrocytes that responded to sinusoidal drifting gratings and were tuned to specific orientations. Natural movies (NM) are known to evoke sparse, but reliable, responses from V1 pyramidal neurons. Surprisingly, we found discrete processes of astrocytes also respond reliably to natural movies. Responses to sinusoidal gratings were also less reliable than to natural movies. We hypothesize that these reliable astrocytic microdomain Ca^{2+} transients are due to the synchronous activation of neighboring ensembles of synapses. Together our results suggest that astrocytes could play an important role in modulating information processing in V1, potentially by modulating response reliability at pyramidal cell synapses.

Disclosures: **R. Garcia:** None. **R. Rikhye:** None. **M. Sur:** None.

Poster

295. Astrocyte Cell Biology and Modulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 295.30/B55

Topic: B.11. Glial Mechanisms

Support: CIHR

Heart and Stroke Foundation

Title: Modulators of the activity threshold for evoked astrocyte Ca^{2+} signals during neurovascular coupling

Authors: *A. INSTITORIS[1], G. R. GORDON[2];
[1]Univ. Of Calgary, Calgary, AB, Canada; [2]Univ. of Calgary, Calgary, AB, Canada

Abstract: Ca^{2+}-dependent pathways in neurons and astrocyte endfeet initiate changes in arteriole diameter to regulate local brain blood flow. We have described that there exists a threshold of synaptic activity that must be met before astrocytic Ca^{2+} elevation can be observed during neurovascular coupling. Although other work has shown that 'supra-threshold' astrocyte activation was the result of synapse-borne glutamate spillover, the factors that set the sensitivity of astrocytes (i.e. change the threshold) to increasing synaptic activity is unknown. Here we test various candidates that may tune the astrocyte activation threshold such as neuromodulators (noradrenaline and acetylcholine), metabolic substrates (oxygen, lactate and adenosine) and extracellular potassium. We used two-photon fluorescence microscopy to examine synaptically evoked synthetic or genetic Ca^{2+} indicator signals around penetrating arterioles in acute slices of the rat neocortex. Our preliminary data exploring the role of extracellular $[K^+]$ found that

although spontaneous Ca^{2+} transients were suppressed by higher external [K^+] (5mM), activation threshold of astrocyte soma and endfeet was not different from that of physiological [K^+] (2.5mM). Our continued work in this area will explore the remaining candidates.

Disclosures: A. Institoris: None. **G.R. Gordon:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.01/B56

Topic: B.11. Glial Mechanisms

Support: NSF Grant 1353739

HHMI

Swiss National Science Foundation Grant 31003A_132969

Microscopy Society of America

University of Arizona Honors College

a private foundation

Title: *Drosophila* astrocytes transport glutamate at identified synapses

Authors: *S. E. MACNAMEE[1], K. E. LIU[1], S. GERHARD[2,3], R. D. FETTER[2], L. P. TOLBERT[1], A. CARDONA[2], L. A. OLAND[1];
[1]Univ. of Arizona, Tucson, AZ; [2]HHMI Janelia Res. Campus, Ashburn, VA; [3]Inst. of Neuroinformatics, Univ. of Zurich and ETH Zurich, Zurich, Switzerland

Abstract: The neuronal circuits that generate behaviors are actively modulated by numerous factors, including the rate at which neurotransmitter transport occurs in neighboring neuronal and glial cells. Details of the neuronal circuit map of the *Drosophila* larval ventral nerve cord are developing rapidly, so we decided to use this model system to probe the effects of altered astrocyte physiology on neuronal circuit output. Here, we examined the anatomical and physiological relationships between *Drosophila* astrocytes and a defined population of pre-motor synapses. We identified a group of neurons with dvGlut-positive terminals that form inhibitory pre-motor connections. These neurons have been named “loopers”, after the looped morphology of their primary process, or “period-positive median segmental interneurons” (Kohsaka et al., 2014). First, we ascertained the distance between looper-motor neuron synapses and astrocyte processes because the temporal dynamics of astrocyte modulation of synaptic transmission depend, in part, on distance. To address this, we used a serial electron micrograph (EM) image

set extending through ventral nerve cord segments A2 – A5. Looper neurons were identified by the ventro-medial location of their cell bodies and the distinct morphology of their primary process in the CATMAID (Collaborative Annotation Toolkit for Massive Amounts of Image Data) environment. We then selected small 3D EM volumes encompassing distal synapse-rich segments of looper neurons and exported them into Reconstruct software. Looper neuron processes, their associated pre-synaptic active zones, and all astrocyte processes within the 3D volume were traced and reconstructed, and the distance between an active zone and the nearest astrocyte membrane was measured. Many looper synapses had no apposing astrocyte processes, and the distance separating active zones from the nearest astrocytic process varied one-hundred-fold from 0.01 to 0.99 microns. Despite this variation in distance, whole-cell recordings from astrocytes revealed that optogenetically-mediated looper activation induces in the astrocytes an inward current with a peak amplitude of 14.4 pA +/- 5.1 SD with delay of approximately 25 ms. 86% of the peak current amplitude was blocked by bath application of the glutamate transporter blocker DL-threo-β-benzyloxyaspartate (DL-TBOA). We currently are examining the effects on motor neurons of pharmacological as well as genetic astrocyte manipulation, allowing us to generate a robust model of astrocyte-synapse modulation.

Disclosures: **S.E. Macnamee:** None. **K.E. Liu:** None. **S. Gerhard:** None. **R.D. Fetter:** None. **L.P. Tolbert:** None. **A. Cardona:** None. **L.A. Oland:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.02/B57

Topic: B.11. Glial Mechanisms

Support: NSF Grant 1353739

Microscopy Society of America

University of Arizona Honors College

a private foundation

Title: Modulation of neural activity affects astrocyte morphology in *Drosophila*

Authors: J. CHARLTON, C. TRAN, S. MACNAMEE, L. P. TOLBERT, *L. A. OLAND; Dept. of Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: Astrocytes are known to maintain a proper ionic balance in the central nervous system, take up neurotransmitters after synaptic signaling, and modulate synaptic activity - and much of this is done through a reciprocal conversation in which neuronal activity induces responses in glial cells that, in turn, modulate neuronal activity. Our long-term goal is to

elucidate the significance and underlying mechanisms of neuron-glia interactions using pharmacological and genetic alterations of neuronal activity in *Drosophila* melanogaster. We previously reported that constitutive RNAi knockdown of the GABA transporter in astrocyte-like glia of the 3rd-instar ventral nerve cord (VNC) strongly reduced larval locomotion, indicating that altering astrocyte function modulates activity in neurons comprising the motor circuitry (MacNamee et al., SfN abstract 2013). Here we have manipulated neural activity in the larva in two ways, to assess the impact of altered neuronal activity on astrocytes. In one set of experiments, we fed larvae picrotoxin, which blocks GABAA receptors and glutamate-gated chloride channels; we used a FLP-out genetic construct to visualize the detailed morphology of astrocytes in the VNC. Results to date indicate that the pharmacological treatment, which would be expected to change the balance of inhibition and excitation, significantly reduced locomotor activity and concomitantly led to a marked decrease in the volume occupied by the branching processes of astrocytes. In a second set of experiments, we used the Q system to overexpress in all neurons a temperature-sensitive allele of shibire to reduce synaptic transmitter release; in the same flies, we used the GAL4/UAS system to drive expression of membrane-targeted green fluorescent protein in astrocytes. We developed a heat-shock protocol that maximizes the period of diminished neural activity, maximizes larval survival, and minimizes time for possible compensatory changes in astrocytes. Overexpression of shibire dramatically reduced larval locomotion. The impact on glial morphology is being assessed using 3-D reconstructions of high-magnification confocal microscopic images.

Disclosures: **J. Charlton:** None. **C. Tran:** None. **S. MacNamee:** None. **L.P. Tolbert:** None. **L.A. Oland:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.03/B58

Topic: B.11. Glial Mechanisms

Support: NSF Grant 1353739

Microscopy Society of America

University of Arizona Honors College

a private foundation

Title: Morphological analysis of astrocyte-like glial cells in the *Drosophila* ventral nerve cord

Authors: E. HERNANDEZ, K. LANCE, J. CHARLTON, S. MACNAMEE, L. A. OLAND, *L. P. TOLBERT;
Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: Understanding the ongoing signaling between neurons and glial cells requires a detailed analysis of basic glial cell morphology and physiology. In particular, the shapes and distribution of glial cells help to dictate the roles those cells play in regulating neuronal function. In each segment of the ventral nerve cord of *Drosophila* melanogaster, the neuropil is organized into distinct motor, interneuron, and sensory neuropils. Each hemisegment of the neuropil is served by just six astrocyte-like glial cells, with cell bodies in characteristic positions around the edge of neuropil. We are asking: What are the detailed morphological features of these astrocytes, and how are their processes arrayed in the synaptic neuropil? We have combined the GAL4/UAS and FLP/FRT systems to stochastically express green fluorescent protein (GFP) in individual astrocytes in the ventral nerve cords of 3rd-instar larvae. For background staining, all astrocyte nuclei were labeled using either an anti-Repo antibody or mCherry targeted to astrocytes, and major axonal tracts (serving as fiducial markers) were visualized with an antibody to Fasciclin II (Fas II). Images were collected on a laser scanning confocal microscope and reconstructed in 3-D. The detailed morphology of each labeled astrocyte and its location within the neuropil were analyzed. 3-D images of 42 individual cells revealed a variety of cell morphologies: some cells are compact with highly branched arbors, some have multiple highly branched arbors, and others have branches that extend out from the compact arborization to wrap around Fas II-positive tracts. To our surprise, 45% of the astrocytes have a process that crosses the midline, 36% have a single thin, unbranched process that travels along the outer rim of the neuropil to a nearby glial cell body, and 9% have a single thin process that leaves the CNS in a peripheral nerve. In separate experiments, we used the GAL4/UAS Flybow system to stochastically label adjacent glial cells with different fluorophores. Overall, we found that astrocytes roughly “tile” the neuropil (as previously noted by Stork et al. 2014); however, the distal processes of adjacent cells often overlap to varying degrees.

Disclosures: E. Hernandez: None. **K. Lance:** None. **J. Charlton:** None. **S. MacNamee:** None. **L.A. Oland:** None. **L.P. Tolbert:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.04/B59

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01ES03299

NIH Grant R25NS54467

NIH Grant T32GM092715

Title: Comparative effects of methylmercury on type 1 cerebellar and cortical astrocytes

Authors: *R. JAIMAN, W. D. ATCHISON;
Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Although methylmercury (MeHg) primarily affects neurons, especially the granule cells in the cerebellum, astrocytes are also targets of this environmental neurotoxic metal. MeHg-induced neurotoxicity in astrocytes has been studied in the cortical layer. However, effects on cerebellar astrocytes are less studied, and regional differences can occur in astrocytes between the two areas. The goal of this study was to compare the toxicity of MeHg on Type 1 cerebellar and cortical astrocytes. Effects on MeHg on Type 1 astrocytes are especially important, because they modulate cell functions in the synaptic cleft and MeHg affects synaptic function of cerebellar granule cells. Determining if MeHg differentially affects Type 1 astrocytes from these two brain regions could help delineate the cellular mechanisms underlying the preferential sensitivity of cerebellar granule cells to MeHg. Primary astrocyte cultures from the cerebellum and cortical forebrain layer were obtained from C57BL/6 male mice. At 13-15 DIV, cells were exposed for 3h to 0μM, 1μM, 2μM, or 5μM MeHg. Cytotoxicity was measured 24h later using EthD-1 and Calcein AM. Type 1 astrocytes were identified immuncytochemically using GFAP and A2B5. Type 1 astrocytes were GFAP+/ A2B5-. In parallel cell cultures, GFAP and the nuclear fluorogenic indicator DAPI were used to calculate the percentages of astrocytes in the cultures. The percentages in the cerebellar and cortical cell cultures were 98% and 100% respectively. The mean percentage of cell death in the cerebellum was: 0 %, 21%, 63%, and 95% at 0, 1, 2, or 5μM MeHg respectively. In the cortical layer it was: 1.1%, 1.3%, 20%, and 73% respectively. Cell viability of cerebellar astrocytes was significantly reduced at 2-5 μM MeHg; in cortical astrocytes viability was reduced only at 5μM. At low MeHg concentrations, cortical astrocytes were less affected by MeHg; the effect was significant at 2 μM MeHg when cerebellar and cortical astrocytes were compared. The LC50s of the cerebellum and cortical layers were 1.15 μM and 4.24 μM MeHg, respectively. In conclusion, Type 1 cerebellar astrocytes are more susceptible to MeHg-induced cytotoxicity than Type 1 cortical astrocytes and brain region-dependent differences in cytotoxicity of astrocytes occur in response to MeHg.

Disclosures: R. Jaiman: None. **W.D. Atchison:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.05/B60

Topic: B.11. Glial Mechanisms

Title: Neuronal activity controls astrocyte proliferation through NMDA receptor signaling

Authors: ***Y. CHEN**[1,2], M. WEBER[1], F. CHU[1], Z. MODRUSAN[1], J. KAMINKER[1], M. SHENG[1];
[1]Genentech INC, Roche Group, South San Francisco, CA; [2]Ircbc, Chinese Acad. of Sciences, SIOC, Shanghai, China

Abstract: Astrocytes are the most abundant cell type in the brain with critical roles in facilitating synapse/circuitry development and functions. But the underlying mechanism that regulates astrocyte proliferation has not yet been demonstrated. We hereby report a surprising result that astrocyte proliferation is bi-directionally regulated by neuronal activity via NMDA receptor (NMDAR) signaling. Using whole genome mRNA profiling, we found that a significant number of cell cycle related genes were altered by the treatment with an NMDAR antagonist in dissociated hippocampal cultures. Interestingly, these cell cycle related genes were expressed and regulated specifically in astrocytes. Furthermore, NMDAR inhibition blocked baseline astrocyte proliferation both *in vitro* and *in vivo*. And evoked neuronal activity was sufficient to promote astrocyte proliferation through NMDARs, suggesting NMDAR signaling could bi-directionally control astrocyte proliferation. Additional mechanistic studies identified an NMDAR-induced molecule, expressed only in neurons, was responsible for this process. In summary, we have found a novel NMDAR-initiated trans-cellular signaling event that controls astrocyte proliferation.

Disclosures: **Y. Chen:** A. Employment/Salary (full or part-time):; Genentech Inc. **M. Weber:** A. Employment/Salary (full or part-time):; Genentech Inc. **F. Chu:** A. Employment/Salary (full or part-time):; Genentech Inc. **Z. Modrusan:** A. Employment/Salary (full or part-time):; Genentech Inc. **J. Kaminker:** A. Employment/Salary (full or part-time):; Genentech Inc. **M. Sheng:** A. Employment/Salary (full or part-time):; Genentech Inc..

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.06/B61

Topic: B.11. Glial Mechanisms

Support: Laboratory of Medical Neurobiology and Electrophysiology, Department of Basic Medical, School of Medicine, Hangzhou Normal University, Zhejiang, China

Institute of Development and Regeneration, Hangzhou Normal University, Zhejiang, China

The project supports from Natural Science Foundation of China

Title: Astrocytes affect the electrical activities of neurons to sound in medial geniculate body

Authors: ***X. SUN**[1], M. HUANG[2];
[1]Hangzhou Normal Univ., Zhejiang, China; [2]Hangzhou Normal Univ., Hangzhou, China

Abstract: Astrocyte(AS)involved in the plasticity of several neurons. But little is known about the characteristics of AS in the auditory processing system. Here we investigated the contributions of astrocyte to the plasticity of medial geniculate body (MGB). We used extracellular electrophysiological recording techniques to record the changes of electrical activities of neurons in MGB. SD rats with unilateral cochlear ablation at different survival time were stimulated by pure tones of different frequency. Before and after the fluorocitrate injection, the electrical activities of the neurons in MGB were recorded. We find that 3 hours after fluorocitrate injection (1) the First spike latency (FSL) was shortened in 24 hours cochlear ablation group (ab24); but for ab14 group the FSL is significantly extended. While FSL of ab30 group exhibited a continuous prolong, we find that ab14 group showed a longer FSL. (2) After some reduction of the spike counts, the non-unilateral cochlear-ablation group (non-ab) showed an increase of spike counts after 2 hours fluorocitrate injection. The spike counts of ab24 and ab14 groups reduced after fluorocitrate injection; and the spike counts of ab30 days group showed an increase first then reduce after fluorocitrate injection. The spike counts of ab24h, ab14d and ab30d were all at their least after 2 hours fluorocitrate injection. The spike counts of ab14d showed a significantly less in all of the groups. (3) According to the frequency tuning curve (FTC), we found that all the characteristic frequency (CF) of non-ab, ab24, ab14 and ab30 group had a tendency moving to the high-frequencies. The minimum threshold (MT) of non-ab group increased first then reduced after fluorocitrate injection; the MT of ab7 and ab30 groups kept increase. The MT of ab24 showed no significant changes to fluorocitrate injection. Our results showed that the functional changes of AS can affect the response to sound of MGB neurons after unilateral cochlear ablation; and the effects are related with the time points of unilateral cochlear ablation. We suggest that AS involved in the plasticity of neurons in medial geniculate body (MGB) to adopt the modulation of auditory processing.

Disclosures: **X. Sun:** None. **M. Huang:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.07/B62

Topic: B.11. Glial Mechanisms

Title: NF-κB-mediated regulation of astrocyte-secreted signals affects the neurogenic potential of adult hippocampal neural progenitors

Authors: V. BORTOLOTTO[1], S. CVIJETIC[1], M. MANFREDI[2], E. RANZATO[3], E. MARENGO[3], P. L. CANONICO[1], *M. GRILLI[4];
[1]DSF, Univ. of Piemonte Orientale Amedeo Avogadro, Novara, Italy; [2]ISALIT srl, DISIT, Univ. of Piemonte Orientale Amedeo Avogadro, Alessandria, Italy; [3]DiSIT, Univ. of Piemonte Orientale Amedeo Avogadro, Alessandria, Italy; [4]Univ. of Piemonte Orientale Amedeo Avogadro, Novara, Italy

Abstract: The hippocampal SubGranular Zone (SGZ) is characterized by the presence of the neurogenic niche, a highly specialized microenvironment which is both instructive and permissive for adult neural progenitor cells (NPC) and their progeny. In previous work we proved that members of the NF-kappaB family of transcription factors are important contributors of signalling pathways in the SGZ neurogenic niche. Within the family, the p50 subunit appears to play a crucial role since p50KO mice display dramatically reduced adult hippocampal neurogenesis in association with short-term memory defects. However, when adult NPC derived from wt and p50KO mice are cultured *in vitro*, no significant differences can be observed in their neurogenic potential, suggesting a potential contribution of other cell subpopulations within the niche to defective neurogenesis in mutant mice. To this purpose we have set up enriched astrocyte cultures from hippocampi of p50KO and wt neonatal mice and studied their influence on wt or p50KO NPC by using astrocyte-conditioned medium (ACM). When wt NPC where exposed to wt ACM, an increased rate of differentiation towards both neuronal and astroglial lineages was observed, in comparison with standard medium. Conversely, p50KO ACM significantly increased the percentage of newly generated astrocytes, but lacked proneurogenic effects on wt NPC. Moreover, wt and p50KO ACM promoted neither neurogenesis nor gliogenesis in p50KO NPC. We then decided to actively pursue the identification of proneurogenic and/or antineurogenic signals that, under control of NF-kappaB p50, may be differentially regulated in astrocytes. To this purpose we analyzed wt and p50KO secretome through a high throughput label-free protein quantitation method called SWATH-MS which allows the relative determination of secreted proteins so to identify molecules differentially modulated in absence or presence of p50. Altogether our data suggest that neurogenic defects observed *in vivo* in p50KO mice are both cell autonomous and non-cell autonoumous, and that they may also involve phenotypic changes in the secretome of astroglial cells. In addition, they add another level of complexity to the role of NF-kappaB p50 in the regulation of adult hippocampal neurogenesis.

Disclosures: V. Bortolotto: None. **S. Cvijetic:** None. **M. Manfredi:** None. **E. Ranzato:** None. **E. Marengo:** None. **P.L. Canonico:** None. **M. Grilli:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.08/B63

Topic: B.11. Glial Mechanisms

Support: NSERC Establishment Grant 195814317

Title: Are astrocytes involved in extending serotonin-mediated neuromodulatory actions to every synapse?

Authors: E. QUON, *L. K. BEKAR;
Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Single astrocytes can ensheath over 100,000 synapses within their domain. Thus, astrocytes are ideally positioned to integrate signals from a few synapses to have impact on all ensheathed synapses with high efficiency. As neuromodulators are released in a volume manner and are known to illicit astrocyte calcium responses, we hypothesized that astrocytes may be effector cells, extending neuromodulator action to every synapse. Using live mouse brain slices and extracellular recordings of evoked excitatory postsynaptic potentials (eEPSPs), we assessed pharmacologically the astrocytic involvement in serotonin-mediated shaping of a simple cortical network containing both excitatory and inhibitory activity. Using a paired-pulse stimulus repeated every 20 seconds, serotonin was administered as a bolus to the bath perfusate upstream of the recording site in order to assess transient effects on the network. To assess the astrocytic role in serotonin effects, serotonin was applied both before and after bath application of pharmacological agents considered to affect astrocyte function or signaling mechanisms. In the absence of neuromodulators or pharmacological agents the first eEPSP is much larger in amplitude than the second due to the recruitment of longer-lasting inhibitory activity resulting from the first stimulus. Pharmacological disruption of gap junctions/hemichannels or impairment of astrocyte metabolism resulted in a significant loss of evoked inhibition in field recordings, suggesting that astrocytes may play a role in tonic aspects of network inhibition. Furthermore, serotonin effects on frequency transmission in the cortical network are significantly lost following so-called astrocyte pharmacological disruption. Lastly, serotonin-mediated frequency transmission could also be disrupted using P2 antagonists suggesting that ATP signaling (astrocyte currency) may be involved. These data highlight the possibility for astrocyte involvement in cortical inhibitory activity seen in this simple cortical network and that serotonin acts on astrocytes to partially exert its modulatory influence.

Disclosures: E. Quon: None. **L.K. Bekar:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.09/B64

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01 HD67218

Nebraska EPSCOR

Title: Role of astrocytic Ca^{2+} signaling in structural synaptic plasticity

Authors: *P. RAGUNATHAN, Y. JUNG, A. DUNAEVSKY;
Developmental Neurosci., Munroe-Meyer Institute, Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Astrocytes are in intimate structural relationship with synapses and astrocytic contact with dendritic spines can promote their stability. Astrocytes display activity-mediated Ca2+ responses and Ca2+ signaling in astrocytes is thought to be involved in astrocyte-neuron signaling possibly through release of gliotransmitters. Astrocytes have therefore been implicated in regulating synaptic transmission and long-term potentiation. It has been shown recently in slices that increased Ca2+ signaling regulates motility of astrocytic processes leading to an enhanced astrocytic coverage of spines and their stability. Whether astrocytic Ca2+ signaling *in vivo* promotes spine stability has not been examined. In order to determine the relationship between astrocytic Ca2+ signaling and dendritic spine structural plasticity, we use the hM3Dq DREADD designer receptor system to selectively stimulate astrocytic Gq-GPCR signaling *in vivo*. Adeno-associated viral injection for expression of hM3Dq in astrocytes was performed in mice expressing GCaMP3 in astrocytes and cranial windows were implanted. Oscillatory-like Ca2+ signals were repeatedly elicited in astrocytes expressing hM3Dq by intraperitoneal injection of CNO (0.025-0.05 mg/kg). Acute and lasting effects of enhanced astrocytic Ca2+ signaling on dendritic spine turnover are being examined by repeated *in vivo* imaging. Understanding the role of astrocytic Ca2+ activity in structural and functional synaptic plasticity is important as this could represent one of the mechanisms by which astrocytes participate in the cellular processes of learning and memory.

Disclosures: P. Ragunathan: None. **Y. Jung:** None. **A. Dunaevsky:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.10/B65

Topic: B.11. Glial Mechanisms

Support: MIUR PRIN 2010-2011

UNIV PD Progetto Ateneo 2012

Telethon Italy GGP14234

Title: Reduced rate of glutamate clearance by astrocytes at cortical excitatory synapses in a mouse model of familial hemiplegic migraine type 2

Authors: C. CAPUANI[1], A. TOTTENE[1], G. CRIVELLARO[1], P. PIZZO[1], M. MELONE[2], L. BRIGINA[2], G. CASARI[3], F. CONTI[2], *D. PIETROBON[1];
[1]Univ. Padova, Padova 35100, Italy; [2]Universita' Politecnica delle Marche, Ancona, Italy; [3]Universita' Vita Salute San Raffaele, Milano, Italy

Abstract: Loss-of-function mutations in ATP1A2, the gene encoding the α2 subunit of the Na+/K+-ATPase, cause familial hemiplegic migraine type 2 (FHM2), a rare subtype of migraine with aura. In the adult brain the α2 Na+/K+-ATPase is expressed almost exclusively in astrocytes. The α2 Na+/K+-ATPase protein is reduced to half in the brain of heterozygous FHM2 knockin (KI) mice carrying the human mutation W887R. Interestingly, these mice show a lower threshold for induction and a higher velocity of propagation of experimental cortical spreading depression (CSD), the phenomenon that underlies migraine aura and can initiate the headache mechanisms. The mechanisms underlying facilitation of experimental CSD in FHM2 KI mice are unknown. Given the key role of NMDA receptors in CSD ignition and propagation and the evidence that the α2 Na+/K+-ATPase and the glial glutamate transporters are colocalized in astrocytic processes surrounding cortical glutamatergic synapses, we investigated whether the rate of glutamate clearance by astrocytes is reduced in FHM2 KI mice as a consequence of the reduced density of α2 Na+/K+-ATPases. We measured the glutamate transporter current elicited in layer 1 astrocytes by extracellular stimulation in acute slices of barrel cortex from P22-23 wild-type (WT) and heterozygous W887R KI mice. The time course of the synaptically activated glutamate transporter current, which reflects the rate of glutamate clearance from the perisynaptic extracellular space, was slower in FHM2 KI compared to WT mice; the slowing down of glutamate clearance was larger after a train of action potentials at high frequency than after a single action potential. Immunogold electron microscopy of parietal cortex revealed that in FHM2 KI mice the density of glutamate transporters GLT1 is reduced in astrocytic processes contacting cortical excitatory synapses, and is unaltered in excitatory axon terminals. This suggests that a decreased density of GLT-1 transporters in perisynaptic astrocytic processes contributes to the reduced rate of glutamate clearance produced by the FHM2 mutation. Our data are consistent with the hypothesis that reduced rate of glutamate clearance at cortical excitatory synapses and consequent increased activation of NMDA receptors particularly during intense neuronal activity may contribute to the facilitation of CSD in FHM2.

Disclosures: **C. Capuani:** None. **A. Tottene:** None. **G. Crivellaro:** None. **P. Pizzo:** None. **M. Melone:** None. **L. Brigina:** None. **G. Casari:** None. **F. Conti:** None. **D. Pietrobon:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.11/B66

Topic: B.11. Glial Mechanisms

Support: NIH Grant MH094268

Title: Astrocytic ALDH7A1 dysfunction in the pathophysiology of neuropsychiatric disorders

Authors: *T. E. FAUST[1,2], T. CASH-PADGETT[1], D. WOOD[1], S. ZOUBOVSKY[1], H. JAARO-PELED[1], A. SAWA[1];
[1]Psychiatry, [2]Neurosci., Johns Hopkins Univ. SOM, Baltimore, MD

Abstract: The function of astrocytes as supporting cells in the brain has long been recognized. However, astrocytes have increasingly been shown to affect neuronal signaling through glutamate uptake, release of gliotransmitters, regulation of extracellular K+ levels and maintenance of osmotic balance. Disruption of astrocyte function plays an important role in central nervous system (CNS) disorders. Recent studies have reported that aldehyde dehydrogenase 7a1 (ALDH7A1; also antiquitin) is enriched in astrocytes compared to other CNS cell types, although its function in astrocytes remains unknown. In non-CNS cells, ALDH7A1 plays a central role in the lysine degradation pathway. It also has been shown to protect against certain cell stressors including osmotic pressure and reactive oxygen species. Clinical studies have identified mutations in the human ALDH7A1 gene as the primary cause of pyridoxine-dependent epilepsy, a childhood form of epilepsy treated with high doses of pyridoxine. The loss of ALDH7A1 enzymatic activity in these patients results in buildup of the toxic intermediates piperideine-6-carboxylate and pipecolic acid which react with pyridoxal phosphate, depleting the brain of an important enzymatic co-factor. We found that ALDH7A1 is highly expressed in multiple astrocyte subpopulations in the adult mouse CNS. We have also found that expression of ALDH7A1 is decreased in biopsied neural tissues from patients with schizophrenia (SZ) and in the frontal cortex a mouse model of psychosis (PCP). Clinical evidence is suggestive of co-morbidity between SZ and epilepsy; functionally, these diseases are linked by alterations in neuronal excitability. In SZ, cognitive changes include working memory (WM) and/or frontal functional deficits influenced by oxidative stress. To study how decreased expression of ALDH7A1 may affect signaling in the brain, we have generated a conditional knockout mouse containing a floxed allele of Aldh7a1. Aldh7a1 knockout mice show reduced seizure threshold and deficits in spatial memory, sensorimotor gating, and forced swimming. Metabolomic analysis also revealed deficits in the lysine degration pathway. No change in GFAP reactivity was observed at baseline, but we found evidence of increased oxidative stress in astrocytes and reduced hippocampal parvalbumin immunoreactivity. We hypothesize that ALDH7A1 dysfunction renders astrocytes more susceptible to oxidative and osmotic stress, disrupting their ability to effectively modulate synaptic signaling and disturbing hippocampal excitatory-inhibitory balance. Through dietary intervention, we may attempt to prevent and/or reverse the observed deficits.

Disclosures: **T.E. Faust:** None. **T. Cash-Padgett:** None. **D. Wood:** None. **S. Zoubovsky:** None. **H. Jaaro-Peled:** None. **A. Sawa:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.12/B67

Topic: B.11. Glial Mechanisms

Support: Swedish Medical Research Council

Swedish Brain foundation

Title: Astrocytic modulation of striatal neurotransmission and endocannabinoid-signaling in Wistar rats

Authors: *L. ADERMARK, F. IVARSSON, A. LOTFI;
Addiction Biol. Unit, Gothenburg, Sweden

Abstract: Astrocytes are pivotal for optimal neurotransmission and participate in several aspects of synaptic functions ranging from trophic support to the fine-tuning of neurotransmission. The precise role of astrocytes in regulating synaptic properties and transmission, however, remains to be investigated. Previous research has shown that mice with a genetic deletion of the astrocyte-specific glutamate transporter GLAST display reduced alcohol preference and impaired endocannabinoid signaling at cortico-striatal synapses, indicating that astrocytes might be directly involved in regulating synaptic plasticity. Using slice electrophysiology, the aim of this study was thus to define the role of astrocytes in modulating synaptic activity and plasticity, with special focus on endocannabinoid-signaling in Wistar rats. Our data show that pretreatment with a low concentration of the glutamate transporter blocker DL-TBOA (200nM), which should act selectively on GLAST and GLT-1, slightly depresses evoked excitatory postsynaptic field potentials (fEPSPs) in the limbic prefrontal cortex, nucleus accumbens (nAc), the dorsolateral striatum (DLS) and the dorsomedial striatum (DMS) in acutely isolated brain slices from both juvenile and adult Wistar rats. Pre-treatment with DL-TBOA inhibited the robust depressant effect on fEPSP amplitude induced by the cannabinoid 1 receptor agonist WIN55,212-2 (1μM) in all brain regions tested. Administration of the mGluR2/3 antagonist LY 341495 (20nM) blocked the acute effect displayed by DL-TBOA on fEPSP amplitude, but did not restore the WIN55,212-2-induced depression. In addition, the astrocytic glycine transporter GlyT1 inhibitor Org24598 (200nM), which did not influence evoked fEPSPs, prevented the WIN55,212-2-induced depression in all brain regions tested. The acute effect displayed by ethanol (50mM) on fEPSC amplitude was not affected by pre-treatment of either DL-TBOA or ORG24598 in any brain region. The data presented here suggests that astrocytes are recruited during endocannabinoid-signaling and that astrocytic transporters may regulate neurotransmission and synaptic plasticity mechanisms in both cortical and striatal subregions.

Disclosures: L. Adermark: None. **F. Ivarsson:** None. **A. Lotfi:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.13/B68

Topic: B.11. Glial Mechanisms

Support: ERC Advanced "Astromnesis"

SNSF 31003A-140999

Title: 3D Ca^{2+} imaging provides new insight into the biology of astrocytes

Authors: E. BINDOCCI[1], N. LIAUDET[1], I. SAVTCHOUK[1], C. DÜRST[1], D. BECKER[1], A. AGARWAL[2], D. E. BERGLES[2], *A. VOLTERRA[1];
[1]Univ. of Lausanne, Lausanne, Switzerland; [2]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Recent studies highlight the existence in astrocytes of a highly diverse gamut of Ca^{2+} dynamics, suggestive of complex functional roles. A single astrocyte exhibits Ca^{2+} signals that range (a) spatially: from “microdomain” events, occurring at focal locations (1-4 μm) along processes, to “expanded” events occupying large portions of a process, to events involving also the cell body; (b) temporally: from rapid sub-sec transients to prolonged multi-sec elevations, occurring either asynchronously or in a more coordinated fashion (reviewed in Volterra et al, Nature Rev Neurosci, 15:327-335, 2014). Despite these key advances, understanding of astrocyte biology is still importantly limited by Ca^{2+} activity being sampled from a single focal plane, which unavoidably loses the 3D+time cell context. Astrocytes have intrinsic 3D complexity, with only a small cell portion remaining confined to any given optical plane. Moreover, they lack any clearly defined compartments (like neuronal spines), yet possess multiple processes that contact blood vessels, synapses, and other neighboring cells along variable 3D trajectories. Our goal here has been to capture astrocytic Ca^{2+} activity in its real 3D context of sub-cellular morphology and neighborhood connectivity. To this end, we equipped a two-photon microscope with piezoelectric actuator and acousto-optic deflector. Thereby, using combined Ca^{2+}-sensitive and Ca^{2+}-insensitive indicators, we could image the full volume of single functioning hippocampal astrocytes in adult (P30-60) mouse brain slices exhibiting endogenous firing activity and observe astrocytic Ca^{2+} dynamics in their 3D cell context. In parallel, we developed a dedicated analysis framework able to handle the “big data” generated from 3D+t multispectral acquisitions. As a result, we observed several types of Ca^{2+} events that expanded differentially across the 3D astrocyte. Such events were affected by agents modifying neuronal firing (e.g. TTX and 4-AP). Importantly, a large proportion of this astrocytic Ca^{2+} activity would have been missed or incompletely reported with current 2D approaches, even using the “best case” 2D focal plane. These data suggest that classic somatic recordings, still often used to study and interpret astrocyte biology, do not faithfully reflect or predict the 3D Ca^{2+} activity of an astrocyte. As an example, in our conditions, activity at processes contacting blood vessels was largely dissociated

from somatic activity. In conclusion, our 3D Ca^{2+} imaging approach provides relevant new insight towards correct understanding of astrocyte biology.

Disclosures: **E. Bindocci:** None. **N. Liaudet:** None. **I. Savtchouk:** None. **C. Dürst:** None. **D. Becker:** None. **A. Agarwal:** None. **D.E. Bergles:** None. **A. Volterra:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.14/B69

Topic: B.11. Glial Mechanisms

Support: NIH NINDS R01NS047557

Title: Expansion of astrocytic processes and reduction of extracellular space volume through activation of β-adrenergic receptors in rat visual cortex

Authors: *A. D. SHERPA[1], F. XIAO[1], N. JOSEPH[2], C. AOKI[3], S. HRABETOVA[1]; [1]Dept. of Cell Biol., SUNY Downstate Med. Ctr., Brooklyn, NY; [2]Herrick High Sch., New Hyde Park, NY; [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Previous results using cultured astrocytes indicate that astrocytes respond to β-adrenergic agonists by changing morphology. Within intact neuropil, this response could facilitate synaptic spillover of neurotransmitters and neuropeptides, thereby enhancing interactions among adjacent neurons and astrocyte endowed with corresponding receptors. We hypothesized that β-adrenergic agonist-induced alteration in the morphology of astrocytic processes impacts upon structural parameters of brain extracellular space (ECS). Brain ECS is a narrow interconnected channel that surrounds brain cells. The two macroscopic parameters - volume fraction (α) and tortuosity (λ) govern the spatiotemporal distribution of substances, including metabolites, signaling molecules, and drugs in the ECS. Volume fraction is the ECS volume, relative to the total tissue volume. Tortuosity is a measure of the hindrance that molecules experience in the ECS, compared to a free medium. We tested our hypothesis by assessing the change in morphology of astrocytic processes within acutely prepared visual cortical slices of adult female rats treated with or without a β-adrenergic agonist, DL-isoproterenol (2 μM) (ISO) for 70 min. Electron microscopy (EM) was used to analyze the neuropil ultrastructure, while kept blind of the drug-treatment condition. The total cytoplasmic area (μm2) of astrocytic processes per micrograph (kept constant at 48.43 μm2) increased significantly, relative to control [1.31 + 0.08 (126); mean ± SEM (number of micrographs) for control; 2.02 ± 0.21 (84) with ISO]. The total plasma membrane length (μm) of astrocytic processes per micrograph significantly increased from a control value of 21.47 ± 0.89 (126) to 29.11 + 1.54 (84) with ISO. The total number of astrocytic profiles per micrograph significantly

increased from 14.75 ± 0.55 (126) in the control condition to 19.55 ± 1.07 (84) with ISO. Values of α and λ were measured in visual cortex with ISO using the real-time iontophoretic (RTI) method. The ECS volume decreased from 0.22 ± 0.01 (mean ± SD; n = 17 records in 5 animals) in the control condition to 0.18 ± 0.01 (n = 23 records in 5 animals) with ISO, while tortuosity remained constant at [1.63 ± 0.01 (n = 17 records in 5 animals) for control; 1.62 ± 0.01 (n = 23 records in 5 animals) with ISO]. Since noradrenergic volume transmission is elevated during the awake state, these findings suggest that one way the awake state, boosted by adrenergic signal, decreases α is through increases in astrocytic volume. The decrease in α would increase ECS concentration of neurotransmitter and neuromodulators, which could facilitate neuronal communication in the visual cortex.

Disclosures: **A.D. Sherpa:** None. **F. Xiao:** None. **N. Joseph:** None. **C. Aoki:** None. **S. Hrabetova:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.15/B70

Topic: B.11. Glial Mechanisms

Support: DFG-EXC307

Title: Changes to AMPAR-mediated glutamatergic signaling at neuron-glia synapses in developing corpus callosum

Authors: ***B. T. KULA**[1], A. HOVHANNISYAN[1,2], T.-J. CHEN[1], B. NAGY[1], M. KUKLEY[1]; [1]Neuron Glia Interactions, Werner Reichardt Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]Biol. Sci., Univ. of California, Santa Cruz, CA

Abstract: Majority of oligodendrocytes in rodents rise during first postnatal weeks from oligodendrocyte precursor cells (OPCs) - a population of progenitors widespread through grey and white matter regions of central nervous system. After the end of myelination OPCs remain in the tissue where they occupy repulsion-driven domains. White matter OPCs come into contact with axons and receive glutamatergic synaptic input mediated mainly by AMPA receptors. Up to now electrophysiological properties of axon-OPC connections were investigated in juvenile and adult animals. However, many studies employed different approaches making direct comparisons of these properties difficult. Additionally, morphological changes of OPCs during development are poorly known. Our work aims at addressing those questions in developing mouse corpus callosum. We employed whole-cell voltage clamp combined with axonal electrical stimulation to investigate properties of callosal neuron-OPC signaling at 3 key developmental ages: P8-11 - callosal OPCs are mainly proliferating, myelination is sparse; P19-22 - peak of callosal

myelination; P50-53 - myelination is largely completed. First, we investigated pre- and postsynaptic properties of neuron-OPC synapses. Our results indicate no changes to kinetics of axon-glia currents, a modest increase in their amplitude and a drop in the release probability as the animals progress in age. Additionally, current-voltage relationship showed little rectification at the youngest age, while an increase in rectification was observed at older ages indicating downregulation of the GluA2 subunit. To address changes in OPC morphology we crossed ROSAmT/mG line carrying membrane-tagged tdTomato/EGFP with NG2CreERTM line carrying tamoxifen-inducible Cre recombinase under NG2 promoter. Upon 4-hydroxytamoxifen (4-TM) administration the mT cassette is deleted allowing expression of mG cassette specifically in OPCs and their progeny. Animals were sacrificed 3 days post 4-HT injection. Confocal image stacks were acquired with 63x magnification and individual OPCs were traced and analyzed with Neurolucida software. We observed differences in cell domain sizes, process structure and complexity. Our findings indicate that OPCs change their physiological and morphological properties during callosal development. Remarkably high variability of many investigated parameters suggests that within each age group OPC population constitutes of cells at different stages of maturation.

Disclosures: **B.T. Kula:** None. **A. Hovhannisyan:** None. **T. Chen:** None. **B. Nagy:** None. **M. Kukley:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.16/B71

Topic: B.11. Glial Mechanisms

Support: UWS grant SG20211

Title: Astrocytic K+ buffering as a mechanism to modulate neuronal oscillations

Authors: ***J. W. MORLEY**, Y. BUSKILA;
Univ. of Western Sydney, Sydney, Australia

Abstract: Different behavioural states are associated with different neural network oscillation frequencies (waves) in the brain. Transition between these states is accompanied with changes in neuronal resonance frequency. One of the major questions in neuroscience is how the activity of single neurons, each with a preferred membrane resonance frequency, can lead to network oscillations. It is clear that this is the result of the responses of multiple neurons being synchronised, but it is unclear exactly how this happens. Furthermore, the mechanism underlying the transition between the different oscillation frequencies remains largely unknown. We have explored the potential involvement of astrocytes in the modulation of neural network

oscillations. As astrocytes form a multicellular network through their intracellular connections, they are in a position to convey local modifications from single neurons and spread them through their glial network to modulate neuronal network behaviour. Moreover they can modulate excitability of neurons by changing the concentration of potassium ions [K+] in the extracellular environment. Using selective inhibition of the K+ influx through astrocytes, we show that increased extracellular K+ concentration can modulate the biophysical properties of individual neurons such as excitability, synchronization and oscillation frequency. As astrocytes are the only cells in the brain capable of K+ buffering through their intracellular connections (gap junctions), our study indicates that modulation of their inherent capabilities to clear K+ from the extracellular milieu is a potential target to impact neural oscillations and thus tuning brain waves.

Disclosures: J.W. Morley: None. **Y. Buskila:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.17/B72

Topic: B.11. Glial Mechanisms

Support: The Government Assignment № 6.26.192014/K

The Russian Foundation Fundamental Research, Agreement №14-04-00901 \ 15 from 20/03/2015

Title: Potassium efflux through postsynaptic NMDA receptors suppresses astrocytic glutamate uptake

Authors: *O. TYURIKOVA[1], P.-Y. SHIH[2], L. SAVTCHENKO[3], D. RUSAKOV[3,1], A. SEMYANOV[1];
[1]Neurosci. Ctr. of the Inst. of Biol. and Biomedicine, Univ. of Nizhny Novgorod, Nizhniy Novgorod, Russian Federation; [2]Purdue Univ., West Lafayette, IN; [3]UCL Inst. of Neurol., London, United Kingdom

Abstract: Potassium ions accumulate in the synaptic cleft of glutamatergic synapses during repetitive activity. We have recently demonstrated that the bulk of these ions is contributed by potassium efflux through postsynaptic NMDA receptors (Shih et al., 2013). Potassium mediated depolarization of presynaptic terminal increases glutamate release probability. Here we investigated the effect of potassium accumulation on astrocytic glutamate uptake because glutamate transporters are both electogenic and potassium-dependent. We recorded glutamate transporter currents in CA1 str.radiatum astrocytes of mouse hippocampal slices in response to electrical stimulation of Schaffer collaterals or local glutamate uncaging. Increases in the extracellular potassium concentration from 2.5 mM to 7.5 mM or 20 mM significantly reduced

the amplitude of uncaging induced transporter currents. In addition, we observed substantial potassium mediated membrane depolarization in current-clamped astrocytes. Mimicking this depolarization in voltage-clamp mode reduced the transporter current to the same extent, reflecting the fact that astrocytic depolarization but not potassium dependence of glutamate transporters suppresses glutamate uptake during potassium accumulation. Repetitive stimulation of Schaffer collaterals (5 stimuli at 50 Hz) produced progressively increasing peaks of transporter currents. This increase was suppressed by 50 μM D-APV, NMDA receptor antagonist. This finding is consistent with our previous results showing the increase in glutamate release probability due to NMDA receptor mediated potassium efflux. However, we also found that D-APV reduced the progressive increase in the decay time of the transporter currents produced by repetitive stimulation. This is consistent with NMDA-receptor dependent reduction of glutamate uptake. Our observations were consistent with predictions of a detailed biophysical model. We suggest that the NMDA receptor dependent accumulation of intracleft potassium during repetitive synaptic activity could inhibit local glutamate uptake, which could potentially extend glutamate dwell-time in the synaptic cleft thus boosting glutamate spillover effects. Pei-Yu Shih, Leonid P. Savtchenko, Naomi Kamasawa, Yulia Dembitskaya, Thomas J. McHugh, Dmitri A. Rusakov, Ryuichi Shigemoto, Alexey Semyanov Retrograde Synaptic Signaling Mediated by K^+ Efflux through Postsynaptic NMDA Receptors / Cell Reports 2013 5:941-951

Disclosures: O. Tyurikova: None. **P. Shih:** None. **L. Savtchenko:** None. **D. Rusakov:** None. **A. Semyanov:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.18/B73

Topic: B.11. Glial Mechanisms

Support: Health South East region in Norway

Title: Subcellular distribution of aquaporin-4 in the human cortex: a study using immunogold electron microscopy

Authors: V. A. EIDSVAAG[1,3], R. ENGER[3,2], A. E. THOREN[3], K. HEUSER[2], P. K. EIDE[1,4], *E. A. NAGELHUS[5,2];
[1]Dept. of Neurosurg., [2]Dept. of Neurol., Oslo Univ. Hosp., Oslo, Norway; [3]Inst. of Basic Med. Sci., [4]Inst. of Clin. Med., [5]Univ. of Oslo, Oslo, Norway

Abstract: Aquaporin-4 (AQP4), the most abundant water channel in brain, plays a crucial role in brain water homeostasis. In mice, AQP4 expression is restricted to ependymal cells and astrocytes, being highly concentrated in astrocytic endfoot membranes at the brain-blood and

brain-cerebrospinal fluid interfaces. The subcellular distribution of AQP4 in the human cortex is poorly characterized. Here we performed a high resolution immunogold analysis of AQP4 expression in non-lesional cortical tissue resected from 12 patients with temporal lobe epilepsy (n=9), aneurism (n=2) or tumor (n=1), and compared data with those obtained in adult mice. Immediately after resection the tissue was immersed into a fixative containing 4% paraformaldehyde and 0.25% glutaraldehyde. Tissue blocks were subjected to freeze substitution, embedded in Lowicryl HM20 resin and processed for postembedding immunogold cytochemistry. In human subjects the AQP4 distribution pattern mimicked that of mice, but showed much higher immunogold labeling densities over perivascular astrocytic endfoot membranes. We present AQP4 immunogold data for endfeet at different segments of the vasculature and compare AQP4 polarization between the two species.

Disclosures: V.A. Eidsvaag: None. **R. Enger:** None. **A.E. Thoren:** None. **K. Heuser:** None. **P.K. Eide:** None. **E.A. Nagelhus:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.19/B74

Topic: B.11. Glial Mechanisms

Support: Health South-East Health Region of Norway

Research Council of Norway

EastDeutsche Forschungsgemeinschaft (STE 552/3)

European Union (ESF EuroEPINOMICS)

Title: Abnormal astrocytic Ca^{2+} signaling in the sclerotic hippocampus of awake mice: a two-photon imaging study using the intracortical kainate injection model of mesial temporal lobe epilepsy

Authors: *R. ENGER[1,4], K. HEUSER[4], C. NOME[4], W. TANG[2], V. JENSEN[2], P. J. HELM[2], K. VERVAEKE[3], P. BEDNER[5], C. STEINHÄUSER[5], E. TAUBØLL[4], E. A. NAGELHUS[2]; [2]Inst. of Basic Med. Sci., [3]Dept. of Biosci., [1]Univ. of Oslo, Oslo, Norway; [4]Dept. of Neurol., Oslo Univ. Hospital, Rikshospitalet, Oslo, Norway; [5]Univ. of Bonn, Inst. of Cell. Neurosciences, Bonn, Germany

Abstract: A growing body of evidence links astrocytes to epileptogenesis and epilepsy. Astrocytic Ca2+ signals are elevated several days after status epilepticus and could contribute to neuronal death as well as seizure formation by facilitating Ca2+ dependent glutamate release. The aim of this study was to characterize astrocytic Ca2+ dynamics in mice with chronic

epilepsy. We used the unilateral intracortical kainate injection model of mesial temporal lobe epilepsy (Bedner P et al., Brain 2015 May;138(Pt 5):1208-22) and *in vivo* two-photon microscopy. Non-injected and sham operated mice served as controls. Three months after kainate injection the genetically encoded Ca2+ indicator GCaMP6f was delivered by recombinant adeno-associated virus (rAAV) injection, using the GFAP promoter to drive expression in astrocytes. Subsequently chronic cranial windows to the CA1 region of the hippocampus were made, enabling repetitive two-photon imaging recordings of astrocytic Ca2+ dynamics in awake, head-fixed mice resting and running on a spherical treadmill. Initial observations revealed a highly abnormal astrocytic Ca2+ signaling pattern in the sclerotic, epileptic hippocampus compared to the hippocampus of control mice. Specifically, we observed a prominent increase in basal Ca2+ fluctuations, as well as frequent, long-lasting Ca2+ transients of high amplitude that involved whole astrocytic territories. In some cases Ca2+ waves spread to several neighboring astrocytes. We conclude that astrocytic Ca2+ dyshomeostasis is a prominent feature in chronic experimental mesial temporal lobe epilepsy.

Disclosures: **R. Enger:** None. **K. Heuser:** None. **C. Nome:** None. **W. Tang:** None. **V. Jensen:** None. **P.J. Helm:** None. **K. Vervaeke:** None. **P. Bedner:** None. **C. Steinhäuser:** None. **E. Taubøll:** None. **E.A. Nagelhus:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.20/B75

Topic: B.11. Glial Mechanisms

Support: JSPS 24590114

Title: Lactate dehydrogenase is an electrical regulator in astrocyte-neuron lactate shuttle of the hippocampus

Authors: ***T. INOUE**, N. SADA;
Okayama Univ., Okayama, Japan

Abstract: Lactate dehydrogenase (LDH) is an enzyme located in the astrocyte-neuron lactate shuttle, a metabolic pathway that transports lactate from astrocytes to neurons in the brain. In the lactate shuttle, glucose is first transported into astrocytes, and converted to lactate via LDH. The lactate is then released and transported into neurons, and converted to pyruvate via LDH. In this study, we examined effects of LDH inhibition on membrane potentials in CA1 pyramidal cells, using patch-clamp recordings from hippocampal slices. Our recordings revealed that pyramidal cells were hyperpolarized by bath application of oxamate, an LDH inhibitor. The hyperpolarization was reversed by the downstream metabolite (pyruvate), but not by the

upstream metabolite (lactate). In contrast to pyramidal cells, the hyperpolarization caused by LDH inhibition was not observed in inhibitory fast-spiking cells. EPSCs in pyramidal cells were also reduced by LDH inhibition, whereas IPSCs were hardly changed. Thus, the excitatory components (pyramidal cells and EPSCs) in the hippocampus are strongly responsive to LDH inhibition. We then made double patch-clamp recordings from pyramidal cells and astrocytes, and applied the LDH inhibitor only into astrocytes via the patch pipette. The LDH inhibition in astrocytes hyperpolarized neighboring pyramidal cells. The hyperpolarization was not observed in the presence of extracellular lactate, which further supports electrical regulation by the lactate shuttle. These results show that pyramidal cells in the hippocampus can be electrically regulated by LDH enzymes in the astrocyte-neuron lactate shuttle.

Disclosures: **T. Inoue:** None. **N. Sada:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.21/B76

Topic: B.11. Glial Mechanisms

Support: UNMC Assistantship-Fellowship

Title: Astrocyte glutamate and metabolic abnormalities in Juvenile Batten Disease

Authors: ***M. BOSCH**, T. KIELIAN;
Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is a neurodegenerative lysosomal storage disease caused by a mutation in *CLN3*. Children with JNCL experience progressive visual, cognitive, and motor deterioration with a decreased life expectancy (late teens-early 20s). There is currently no effective treatment for children with JNCL. Studies have shown that astrocyte activation precedes and predicts regions of neuronal loss in JNCL, suggesting alterations in supportive glial functions. Glutamate is elevated in JNCL brains and neuronal loss is hypothesized to result, in part, from glutamate excitotoxicity. Astrocytes are responsible for removing glutamate at the synapse and regulating neuronal activity. Currently, little is known about aberrant glutamate cycling in astrocytes during JNCL progression. Studies conducted in our laboratory utilizing $CLN3^{\Delta ex7/8}$ mice, which harbor a 1.02 kb mutation spanning exons 7 and 8 (the most common mutation found in ~85% of JNCL patients), have revealed a significant decrease in glutamine synthetase in the $CLN3^{\Delta ex7/8}$ brain. Additionally, expression of the glutamate transporter GLAST was significantly reduced in multiple brain regions of $CLN3^{ex7/8}$ mice. From these results, we hypothesized that CLN3 is critical for maintaining glutamate cycling pathways and homeostatic communication between astrocytes and neurons. Recent data

from our laboratory has shown that glutamate transporter expression and function is significantly decreased in primary $CLN3^{ex7/8}$ astrocytes following exposure to stimuli elevated in the JNCL brain (i.e. TNF-α and IL-1β or ceramide and neuronal lysate). A role for CLN3 in astrocyte metabolism was demonstrated by the finding that $CLN3^{\Delta ex7/8}$ astrocytes had significantly lower levels of basal mitochondrial respiration, ATP production, and maximal respiration under resting conditions. These differences were further exacerbated when $CLN3^{\Delta ex7/8}$ astrocytes were treated with TNF-α and IL-1β or ceramide and neuronal lysate. Glutamate regulation is an energy-demanding process and disruptions in metabolic pathways could further disrupt glutamate cycling. Accordingly, our findings revealed decreased Ca^{2+} signaling in $CLN3^{\Delta ex7/8}$ astrocytes that was coupled with heightened $CLN3^{\Delta ex7/8}$ neuron activity, suggesting that aberrant glutamate cycling disrupts vital homeostatic signaling networks. Further elucidation of the key regulatory mechanisms that are involved in astrocyte-neuron cross-talk during JNCL will be critical to understanding disease progression and may ultimately unveil novel therapeutic targets that will extend the quality-of-life for children suffering from JNCL.

Disclosures: M. Bosch: None. **T. Kielian:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.22/B77

Topic: B.11. Glial Mechanisms

Title: Astroglial type-1 cannabinoid receptors (CB1) are necessary for object recognition memory and synaptic plasticity

Authors: *L. M. ROBIN[1,2], J. F. OLIVEIRA DA CRUZ[1,2,3], V. C. LANGLAIS[1,2], A. BUSQUETS-GARCIA[1,2], E. SORIA-GOMEZ[1,2], F. DRAGO[3], A. PANATIER[1,2], F. GEORGES[2], M. METNA-LAURENT[1], S. OLIET[1,2], G. MARSICANO[1,2];
[1]Neurocentre Magendie, Bordeaux Cedex, France; [2]Univ. of Bordeaux, Bordeaux, France; [3]Univ. of Catania, Catania, Italy

Abstract: Astrocytes express a wide variety of G protein-coupled receptors (GPCR) that can influence cognitive functions such as learning and memory. Cannabinoids and endocannabinoids modulate memory processes through the GPCR CB_1 receptor. Similarly to neurons, astrocytes express functional CB_1 receptors capable of modulating the effects of exogenously administered cannabinoid agonists on hippocampal synaptic plasticity and working memory. However, the physiological roles of astroglial CB_1 receptor in long-term memory and synaptic plasticity remain unknown. Here, we show that the conditional genetic deletion of CB_1 receptor in astrocytes (GFAP-CB_1-KO mice) impairs both *in vitro* and *in vivo* hippocampal N-methyl-D-Aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) induction and long-term

object recognition memory. Notably, administration of D-serine, a NMDAR) co-agonist, which is released by astrocytes, restores the long-term memory deficit and the induction of LTP in our mutant mice. Finally, GFAP-CB_1-KO mice present strongly reduced occupancy of the co-agonist binding site of NMDARs. Thus, astroglial CB_1 receptors are necessary for long-term memory and the induction of NMDAR-dependent LTP, via the modulation of the occupancy of the NMDAR co-agonist binding site. This study reveals an unexpected role for astroglial CB_1 receptors in neural information processing and memory formation.

Disclosures: L.M. Robin: None. **J.F. Oliveira da Cruz:** None. **V.C. Langlais:** None. **A. Busquets-Garcia:** None. **E. Soria-Gomez:** None. **F. Drago:** None. **A. Panatier:** None. **F. Georges:** None. **M. Metna-Laurent:** None. **S. Oliet:** None. **G. Marsicano:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.23/B78

Topic: B.11. Glial Mechanisms

Support: Netherlands Organization for Scientific Research Veni grant (NWO 863.12.006)

Title: The role of astrocytic versus neuronal cannabinoid receptors in developmental plasticity of the visual cortex

Authors: *R. MIN[1], B. LUTZ[2], G. MARSICANO[3], C. N. LEVELT[1];
[1]Mol. Visual Plasticity group, Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [2]Inst. of Physiological Chem., Univ. Med. Ctr. of the Johannes Gutenberg Univ., Mainz, Germany; [3]Endocannabinoids and Neuroadaptation, INSERM U862 NeuroCentre Magendie, Bordeaux, France

Abstract: Endocannabinoids are small lipid signaling molecules involved in synaptic plasticity. They are ligands for the cannabinoid CB1 receptor, and play an important role in the postnatal refinement of synaptic connections in the sensory cortex. As a consequence, interfering with endocannabinoid signaling in young animals leads to aberrant sensory map formation. At the synaptic level, endocannabinoids are thought to regulate sensory plasticity by mediating a developmental form of long-term synaptic depression (LTD) at both excitatory and inhibitory synapses. Previous studies have suggested that CB1 receptors on (PV-positive) interneurons in the visual cortex are crucial for a developmental form of LTD of inhibitory synapses (i-LTD). Furthermore, the ability of inhibitory synapses to show i-LTD was suggested to be related to the presence of ocular dominance (OD) plasticity during the critical period in developing visual cortex. Therefore, we tested whether removal of CB1 receptors specifically from interneurons would interfere with critical period OD plasticity. Surprisingly, we found that the OD shift in

primary visual cortex observed after three days of monocular deprivation of the contralateral eye was fully intact in interneuron specific CB1 receptor knockout mice. Interestingly, we and others have recently shown that astrocytes express functional CB1 receptors, and that these astrocyte CB1 receptors are involved in some forms of endocannabinoid mediated LTD. In line with this, we find that astrocytes in the developing visual cortex respond to cannabinoid receptor activation with increased calcium signaling, indicating that they express functional CB1 receptors. We are currently testing whether specific removal of astrocyte CB1 receptors affects OD plasticity and LTD in primary visual cortex. With these experiments we will determine whether neuronal or astrocytic CB1 receptors control developmental visual cortex plasticity.

Disclosures: **R. Min:** None. **B. Lutz:** None. **G. Marsicano:** None. **C.N. Levelt:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.24/B79

Topic: B.11. Glial Mechanisms

Support: Telethon Italy GGP10138

Telethon Italy GGP12265

Cariparo Foundation

CNR Aging Project

FIRB RBAP11X42L

San Paolo "Programma in Neuroscienze"

MIUR FIRB (RBAP11X42L)

Title: Somatostatin and parvalbumin interneuron signalling to astrocytes

Authors: L. MARIOTTI[1], G. LOSI[1], M. SESSOLO[1], I. MARCON[1], S. BOVETTI[2], T. FELLIN[2], *G. CARMIGNOTO[1];
[1]Neurosci. Institute, CNR and Univ. of Padova, 35121 Padova, Italy; [2]Inst. Italiano di Tecnologia, Genova, Italy

Abstract: The reciprocal communication between neurons and astrocytes represents a fundamental mechanism for the control of brain functions. While astrocyte interplay with excitatory glutamatergic neurons has been intensively studied, many fundamental aspects of neuron-astrocyte partnership remain unexplored. Indeed, whether the information gained from the tripartite glutamatergic synapse can be extended to the other large family of synapses in the

brain, the inhibitory GABAergic synapse, is unclear at present. We here reveal that in somatosensory cortex (SSCx) slice preparations, obtained from P15-P22 mice after loading with the Ca2+ indicator Fluo-4 AM and the astrocytic marker SR101, astrocytes exhibited repetitive Ca2+ elevations in response to challenge with GABA or the GABAB receptor agonist Baclofen applied in the presence of Tetrodotoxin (TTX, 0.5 μM). Most importantly, similar responses were observed in astrocytes from the somatosensory cortex of young adult mice (P35-40) in both slice and *in vivo* preparations. We also used optogenetic techniques to investigate the specific response of astrocytes to synaptic GABA release. To this aim, the expression of the light-gated channel channelrhodopsin-2 (ChR2) in Parvalbumin (Pv) or Somatostatin (Som) interneurons was induced by injecting AAV vectors carrying the doublefloxed ChR2 sequence in Pv- or Som-Cre mice at postnatal day 0-2. Blue light illumination (λ=473 nm) was used to selectively activate Pv or Som interneurons expressing virally delivered ChR2. In two-photon laser-scanning microscope experiments in SSCx slice preparations we found that a selective optogenetic activation of Som or Pv interneurons (20÷200 ms pulse duration; 1 Hz for 30÷60s; illuminated area 0.33 mm2; mean power 38 mW/mm2) evoked Ca2+ events in astrocytes at the level of soma, proximal and distal processes. We also investigated whether GABA released from individual GABAergic interneurons can be sufficient to activate neighbouring astrocytes. We found that an intense AP firing induced in individual Pv or Som interneurons by depolarizing intracellular current pulses (200-300 pA for 100 ms at 0.5-1Hz) evoked Ca2+ elevations in neighbouring astrocytes. GABA-mediated inhibition represents a fundamental operational mechanism in the brain and GABAergic interneurons are possibly involved in the pathophysiology of many brain disorders, including epilepsy. The specific signaling between specific GABAergic interneuron populations and astrocytes open up new perspectives in our understanding of the astrocyte role in the brain.

Disclosures: **L. Mariotti:** None. **G. Losi:** None. **M. Sessolo:** None. **I. Marcon:** None. **S. Bovetti:** None. **T. Fellin:** None. **G. Carmignoto:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.25/B80

Topic: B.11. Glial Mechanisms

Support: CIHR (#14392)

Title: Role of astrocytic coupling in rhythmogenesis in rat trigeminal neurons involved in mastication

Authors: *S. CONDAMINE[1], R. LAVOIE[2], A. KOLTA[1];
[1]Dept. de Neurociences, Univ. De Montréal, Montreal, QC, Canada; [2]Douglas Mental Hlth. Res. Inst., Montréal, QC, Canada

Abstract: Neurons in the dorsal part of the trigeminal main sensory nucleus (NVsnpr) have intrinsic bursting properties. Because they receive sensory inputs and project to motoneurons, they have been postulated to form the rhythmogenic core of the masticatory CPG. In absence of Ca2+, bursting is spontaneous in NVsnpr neurons, but in presence of Ca2+ it can be induced by local application of NMDA or electrical stimulation of sensory inputs. Riluzole impairs neuronal bursting induced under all of these conditions, demonstrating the involvement of a voltage-dependent sodium persistent current (INaP), which is modulated by the extracellular Ca2+ concentration ([Ca2+]e). Our previous work shows that S100β, an astrocytic Ca2+ binding protein, decreases [Ca2+]e and induces INaP dependent bursting in neurons. Blockade of S100β or inactivation of astrocytic networks with intracellular dialysis of BAPTA prevents bursting in neurons .These data raise the hypothesis that astrocytic coupling may play an important role in rhythmogenesis. To test this hypothesis, we first investigated whether coupling is modulated by stimuli that induce neuronal bursting in NVsnpr using biocytin filling of astrocyte during patch clamp recordings. Little coupling was observed between NVsnpr astrocytes when no treatment was applied during recording. Networks observed under control conditions (n=14) had an average area of 19374 ± 6973 μm2, and were composed of 9± 3 cells. Electrical stimulation of sensory fibers projecting to NVsnpr induced membrane depolarisation in 11/17 astrocytes tested. Networks associated to responding astrocytes (n=11) had an average area of 35427 ± 13248 μm2 and counted 16 ± 7 cells. Those not showing a depolarisation (6/17) were composed of 8 ± 3 cells and had an average area of 32502 ± 19686 μm2. Local NMDA applications were also associated to large membrane depolarisation and increased coupling (29 ± 3 cells; average area: 85655 ± 15962μm2). Similar increases in coupling were observed when lowering [Ca2+]e (n=31; 32 ± 31cells; average area:55816 ± 49749μm2). All astrocytic networks remained confined to the dorsal part of NVsnpr, and within this area, adjacent networks revealed in double dye filling experiments did not overlap. Bath application of carbenoxolone (20μM), a large connexin inhibitor, inhibited coupling under all conditions and disrupted NMDA-induced neuronal bursting. Bursting was restored by local application of S100β. These results suggest that astrocytes are organized in non-overlapping networks that are regulated by sensory inputs and that these networks are necessary for neuronal bursting.

Disclosures: **S. Condamine:** None. **R. Lavoie:** None. **A. Kolta:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.26/B81

Topic: B.11. Glial Mechanisms

Support: Research Council of Norway

Title: Unique Ca^{2+} responses of subpial and perivascular astrocytic endfeet to hypo-osmotic stress: a two-photon imaging study using acute cortical slices from adult mice

Authors: *M. EILERT-OLSEN[1], V. JENSEN[1], R. ENGER[2], P. J. HELM[1], T. WANNAN[3], A. E. THOREN[1], E. A. NAGELHUS[1,2,3];
[1]Univ. of Oslo, Inst. of Basic Med. Sci., Oslo, Norway; [2]Dept. of Neurol., Oslo Univ. Hospital, Rikshospitalet, Oslo, Norway; [3]Ctr. for Mol. Med. Norway, Univ. of Oslo, Oslo, Norway

Abstract: Being encased in the rigid skull the brain must possess mechanisms for effective volume control. Astrocytic endfoot processes form the outermost layer of nervous tissue and are thus strategically positioned to regulate the movement of solutes and water between the parenchyma and the extracerebral liquid compartments, e.g. cerebrospinal fluid and blood. Astrocytic Ca2+ signals have been implicated in brain volume homeostasis, but endfoot Ca2+ signals are poorly characterized. The advent of ultrasensitive genetically encoded Ca2+ indicators have enabled investigation of Ca2+ signaling in cellular microdomains, including astrocytic endfeet. Here we used two-photon microscopy to assess the Ca2+ response of astrocytic somata, fine processes and endfeet in adult mouse cortical slices subjected to 20% reduction of artificial cerebrospinal fluid osmolarity. Astrocytic Ca2+ signals were detected by GCaMP6f delivered by recombinant adeno-associated virus 2-4 weeks prior to imaging. Lowering external osmolarity elicited a 4-5 fold increase in the frequency of Ca2+ signals in astrocytic endfeet bordering pia mater and ensheathing cortical blood vessels, whereas Ca2+ signaling in fine astrocytic processes within the neuropil was only moderately elevated. In contrast, the frequency of Ca2+ signals in astrocytic somata remained unchanged. The pattern of Ca2+ signals differed between subpial and perivascular endfeet. Specifically, oscillatory Ca2+ signals were only observed in endfeet underneath the pia. In mice deficient in dystrophin, a crucial component of the aquaporin-4 anchoring complex in endfoot membranes, the Ca2+ response to hypo-osmotic stress was significantly reduced only in subpial endfeet. We conclude that astrocytic endfeet at the brain-CSF and brain-blood interfaces display unique Ca2+ responses to hypo-osmotic stress, and that subpial and perivascular endfeet differ in Ca2+ signaling.

Disclosures: **M. Eilert-Olsen:** None. **V. Jensen:** None. **R. Enger:** None. **P.J. Helm:** None. **T. Wannan:** None. **A.E. Thoren:** None. **E.A. Nagelhus:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.27/B82

Topic: B.11. Glial Mechanisms

Title: Volume dynamics of astroglial endfeet during cortical spreading depression in adult mice: an *in vivo* two-photon imaging study

Authors: *D. B. DUKEFOSS[1], B. ROSIC[1], V. JENSEN[1], A. THOREN[1], R. ENGER[1,2], E. A. NAGELHUS[1,2];
[1]Dept. of Mol. Med., Univ. of Oslo, Inst. of Basic Med. Sci., OSLO, Norway; [2]Dept. of Neurol., Oslo Univ. Hosp., Oslo, Norway

Abstract: Cortical spreading depression (CSD) is a slowly propagating wave of almost complete grey matter depolarization followed by temporary EEG silencing. The phenomenon is thought to underlie the migraine aura and is associated with neuronal swelling and profound increase in extracellular glutamate and potassium. During CSD arterioles contract despite that the parenchyma is severely metabolically stressed and in need for enhanced oxygen and glucose supply. The aim of this study was to investigate volume dynamics of perivascular endfeet in CSD, as endfoot volume dyshomeostasis could contribute to vascular uncoupling and tissue metabolic stress. CSD was evoked by focal epidural application of KCl in a distant separate craniotomy to the imaging window. Astrocytic endfeet were imaged in anesthetized mice expressing enhanced green fluorescent protein under the astrocyte specific Glt1-promoter. The vasculature was outlined by Texas Red-labelled dextran. Two-photon microscopy revealed that the initial arteriolar contraction in CSD was accompanied by a profound increase in cross-sectional area of periarteriolar endfeet. Our data suggest that astrocytic endfoot volume dyshomeostasis occurs in CSD.

Disclosures: D.B. Dukefoss: None. **B. Rosic:** None. **V. Jensen:** None. **A. Thoren:** None. **R. Enger:** A. Employment/Salary (full or part-time):; 2Department of Neurology, Oslo University Hospital, Rikshospitalet, Oslo, Norway. **E. A. Nagelhus:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.28/B83

Topic: B.11. Glial Mechanisms

Support: IISER Pune, India

Wellcome Trust/DBT India Alliance

Title: A computational model of purinergic modulation of synaptic transmission by astrocytes

Authors: *A. G. PILLAI, S. NADKARNI;
Computat. Neurobio. Lab., IISER Pune, Pune, India

Abstract: Recent studies suggest ATP release from glial cells as a potential mechanism through which astrocytes are able to influence synaptic transmission. However, the contribution of ATP, to ongoing activity, unlike glutamate, is quite difficult to quantify. This is due to the presence of multiple receptor subtypes which lack specific antagonists and the relatively large diversity in their time profile. In the present study we develop a biophysically detailed model of gliotransmitter release from astrocytes that is in agreement with experimental data. Further we investigate synaptic modulation at a hippocampal synapse by ATP and glutamate released from astrocytes in a computational model of a tripartite synapse. Activity in glutamatergic hippocampal synapses during different behavioral stages range from a fraction of hertz to tens of hertz. This leads to distinct levels of ambient glutamate that can activate astrocytes causing them, in turn, to release gliotransmitters like glutamate and ATP. Via both ionotropic and metabotropic receptors, ATP and adenosine target presynaptic calcium and potassium currents, modulate neuronal excitability and synaptic transmission. Separately, presence of purine receptors in astrocytes sets in a feedback loop that alters IP3 coupled calcium release from these cells. How do these neurotransmitters and gliotransmitters either independently or synergistically regulate synaptic transmission? Our model describes astrocytic modulation of synaptic transmission in response to ongoing 1) Basal 2) Medium 3) High frequency activity. Our computational paradigm gives valuable insights into purinergic modulation of synaptic plasticity by astrocytes.

Disclosures: **A.G. Pillai:** None. **S. Nadkarni:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.29/B84

Topic: B.11. Glial Mechanisms

Support: NIH Grant HS4024

Title: *In vivo* exosome-mediated transfer of microRNA from neuron to astroglia

Authors: ***N. H. BOWENS**, J. YELICK, Y. YANG;
Tufts Univ., Boston, MA

Abstract: Neuron to astrocyte signaling is essential for functional tripartite synapses in the mammalian central nervous system. Accumulating evidence has emerged that miRNAs are packed into exosomes that are secreted into the extracellular space from the multivesicular body. This has been considered a new intercellular communication mechanism. Previous evidence in our lab showed that neurons up-regulate the expression of GLT-1 protein via exosomal transfer of microRNA 124 (miR-124). However, exosome mediated transfer of miRNA has not been demonstrated *in vivo*. Here we investigated miRNA transfer from spinal cord motor neurons to

astrocytes using retrograde transport of labeled miRNAs in astrocyte reporter BAC-ALDH1L1-eGFP and EAAT2-tdTomato transgenic mice. Labeled miRNAs are mixed with fluoro-Gold and are injected into the sciatic nerve of the hind limb. We examined spinal cord sections 7d, 14d, and 21d post-injection. Interestingly, we found labeled miRNAs in extracellular space. Some of the labeled miRNAs are overlapped with eGFP or tdTomato labeled astrocytes. In addition, we performed immunogold EM of cd63, a membrane marker of exosomes on brain sections and identified exosome structure near post-synaptic dendritic spines. Overall, our current study provides *in vivo* evidence for exosome mediated transfer of miRNAs from neurons to astrocytes.

Disclosures: **N.H. Bowens:** None. **J. Yelick:** None. **Y. Yang:** None.

Poster

296. Astrocyte-Neuron Interactions I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 296.30/B85

Topic: B.11. Glial Mechanisms

Support: Canadian Institutes of Health Research

Natural Sciences and Engineering Research Council of Canada

Canada Research Chairs Program

Brain Canada/W. Garfield Weston Foundation

Canadian Foundation for Innovation

Research Institute of the McGill University Health Centre

Title: Neurons diversify astrocytes in the adult brain through Sonic Hedgehog signaling

Authors: *W. T. FARMER[1], T. ABRAHAMSSON[1], S. CHIERZI[1], C. LUI[1], E. JONES[1], B. PONROY[1], J. PENG[2], F. CHARRON[2], P. SJOSTROM[1], K. MURAI[1];
[1]Ctr. for Res. in Neurosci., McGill Univ., Montreal, QC, Canada; [2]Mol. Biol. of Neural Develop., Inst. de Recherches Cliniques de Montréal, Montreal, QC, Canada

Abstract: Astrocytes play key roles in the healthy brain from regulating extracellular neurotransmitter/ion homeostasis and synaptic plasticity to controlling neurovascular coupling. The ability of astrocytes to fulfill such diverse responsibilities relies on their heterogeneous molecular and physiological profiles. However, the source of astrocyte heterogeneity in the mature brain and the mechanisms involved remain poorly understood. We examined gene expression databases for signaling pathways that were differentially expressed in astrocytes to uncover the origin astrocyte heterogeneity. We found that the genes necessary for the reception

of the morphogen Sonic Hedgehog (Shh) were expressed at high levels in specific populations of astrocytes in the adult brain while other astrocyte expressed lower levels. We also found that the secreted ligand Shh is expressed by neurons throughout the adult brain. These expression patterns indicate that the Shh pathway could be a novel mode of neuron to astrocyte communication. To understand the importance of the Shh pathway in regulating the properties of adult astrocytes *in vivo*, we utilized conditional loss- and gain-of-function genetic tools for Shh pathway components coupled with 2-photon imaging, neurotransmitter uncaging, whole-cell electrophysiology, and quantitative RT-PCR (qRT-PCR). We found that increasing and decreasing the activity of the Shh pathway in the mature brain led to bi-directional changes in the expression of key astrocyte molecules that mediate neurotransmitter detection, neurotransmitter uptake, and ion homeostasis. The observed changes in gene expression led to corresponding changes in astrocyte physiology. Interestingly, astrocytes in different brain regions appear to show different responses to activating or eliminating the Shh pathway. Our results demonstrate that mature astrocytes display extensive molecular plasticity in the mature, uninjured brain and the molecular and physiological properties of astrocytes are not hardwired during development but are dependent on neuron-derived Shh. The ability of neurons to control the molecular properties of astrocytes likely ensures that astrocytes are equipped with an optimal molecular toolkit to meet the physiological demands of specific brain circuits. Further work is needed to determine if neuron-astrocyte communication through Shh pathway is disrupted in various brain diseases.

Disclosures: W.T. Farmer: None. **T. Abrahamsson:** None. **S. Chierzi:** None. **C. Lui:** None. **E. Jones:** None. **B. Ponroy:** None. **J. Peng:** None. **F. Charron:** None. **P. Sjostrom:** None. **K. Murai:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.01/B86

Topic: B.11. Glial Mechanisms

Support: NIMH KO1 MH087845

Brain and Behavior Foundation Young Investigator Award to KMS

UL Lafayette Graduate Student Organization

Title: Calcium imaging of cocultured GABAergic interneurons with FGFR1 knock out astrocytes

Authors: *D. J. ROGERS[1], M. JACKSON[2], H. TORRES[2], B. FORET[2], G. WATSON[2], K. M. SMITH[2];

[1]Univ. of Louisiana at Lafayette, Rayville, LA; [2]Biol., Univ. of Louisiana at Lafayette, Lafayette, LA

Abstract: Astrocytes in the cerebral cortex have many functions that support neuronal integrity. One such function is maintaining ionic homeostasis of the extracellular fluid; this may involve the regulation of the ionic environment, release of neurotransmitters or rapid reuptake of excess neurotransmitters. Alterations in astrocyte function could affect the associated neuronal field, in turn, effecting neuronal activity or neuronal survival. We previously published that transgenic *FGFR1*$^{Flox/Flox;NestinCre}$ mice have a decreased number of cortical interneurons. Interneurons grown on *FGFR1*$^{Flox/Flox;NestinCre}$ mouse astrocytes presented smaller soma size and fewer dendritic processes when compared to their littermates. The physiology underlying this morphological phenotype is unknown. One possible hypothesis is *FGFR1*$^{Flox/Flox;NestinCre}$ cortical astrocytes are unable to maintain the proper extracellular environment for proper function and/or survival of these interneurons. A coculture model using P2-P4 *FGFR1*$^{Flox/Flox;NestinCre}$ knockout mice or control littermate astrocytes to form a feeder layer for embryonic medial ganglionic eminence derived *Gad-67 GFP* + labeled GABAergic interneurons was developed to better understand the function FGFR1 has in astrocyte physiology. Techniques using intracellular calcium imaging with Fluro 3-AM, revealed calcium waves in neurons and astrocytes of knock out and control mice. Movies of these waves were analyzed using Image J. The inhibitory interneurons grown on *FGFR1*$^{Flox/Flox;NestinCre}$ knockout astrocytes had significantly fewer calcium peaks when compared to control. (*FGFR1*$^{Flox/Flox;NestinCre}$ knockout astrocytes =4.75 ± 3.38 peaks, *FGFR1*control astrocytes =20.13 ± 3.38, p=0.0063*) An anecdotal point to be further investigated was the oservation that the calcium peaks of cocultured interneurons on the knockout astrocytes were lower amplitude than control. These results suggest that eventhough we have previously shown that there was no significant difference in the calcium amplitude response between the *FGFR1*$^{Flox/Flox;NestinCre}$ knockout and control astrocytes. There are definitely significant differences when interneurons are applied to these astrocytes, indicating the importance of the astrocyte-neuron interaction.

Disclosures: D.J. Rogers: None. **M. Jackson:** None. **H. Torres:** None. **B. Foret:** None. **G. Watson:** None. **K.M. Smith:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.02/B87

Topic: B.11. Glial Mechanisms

Title: Retroaxonal barrage firing in NPY interneurons of the hippocampus: a role for astrocytes

Authors: *T. DEEMYAD[1], N. SPRUSTON[2];
[1]Howard Hughes Med. Institute/Janelia Farm, Ashburn, VA; [2]Howard Hughes Med. Institute/Janelia, Ashburn, VA

Abstract: In CA1 area of the hippocampus, repeated stimulation of NPY-GFP+ GABAergic interneurons leads to a form of persistent firing called retroaxonal barrage (RaB) firing (Sheffield et al. 2011, 2013). We have shown previously that a decrease in extracellular calcium concentration or block of voltage-gated calcium channels inhibits RaB firing. However, intracellular BAPTA in NPY-GFP+ interneurons did not prevent RaB firing. Furthermore, inhibition of gap junctions blocks RaB firing, but gap junctions other than Cx36, the major connexin between CA1 interneurons, are responsible for this effect. These observations led us to the hypothesis that the calcium signaling and gap junctions necessary for RaB firing could be in astrocytes. To explore this hypothesis, we used a combination of electrophysiology, calcium imaging and optogenetics in astrocytes. All experiments were performed in hippocampal slices prepared from the CA1 area of the hippocampus in mice expressing GFP under the NPY promoter in interneurons. Double recordings from astrocytes and NPY-GFP+ interneurons revealed a subset of astrocytes (n=8/28) that depolarized >10 mV near the onset of RaB firing (onset range: 18 sec before to 10 sec after RaB). Enhancing the astrocytic network depolarization by bath application of BaCl2 facilitated RaB firing in all recordings (n=6/6). To further study the relation between calcium transients in the network of astrocytes and RaB firing, we used the genetically encoded calcium indicator, GCaMP3 in astrocytes. While no synchronized calcium activity was observed in the network, many astrocytic processes exhibited increased fluorescence prior to and during RaB firing (onset range: 7-21 sec prior to RaB generation; peak range from onset of RaB: 45-95 sec) and stayed relatively stable for the duration of RaB firing. To investigate the connection between astrocytic calcium signaling and RaB firing, we used intracellular BAPTA for astrocyte recordings. RaB firing was first generated in interneurons and a nearby astrocyte was subsequently patched using pipettes containing BAPTA (50mM). In some cases, this resulted in complete block or an increase in the number of spikes required for induction of RaB firing (n=7/25). Finally, stimulation of astrocytes with channelrhodopsin (ChR2) increased calcium transients in astrocytes and decreased the threshold for RaB firing by ~45% (n=8/12); in some cases ChR2 stimulation was sufficient to generate RaB without depolarizing stimulation. Together, these findings suggest that the astrocytic network plays a direct role in regulating the induction of RaB firing in NPY interneurons.

Disclosures: T. Deemyad: None. **N. Spruston:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.03/B88

Topic: B.11. Glial Mechanisms

Support: NIMH K01MH087845

NARSAD Young Investigator Award BBF

NIMH R01MH067715-01A1

Title: Fibroblast growth factor receptor signaling in astrocytes

Authors: *K. M. SMITH[1], L. B. RUBIN[2], L. CHOUBEY[2], J. COLLETTE[2], R. R. H. DEEGAN[3], F. M. VACCARINO[4];
[2]Biol., [1]Univ. of Louisiana At Lafayette, Lafayette, LA; [3]Child Study Ctr., [4]Child Study Center, Neurol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Fibroblast growth factors (fgfs) are a family of 22 cytokines and 4 receptors, many of which play a critical role in cortical development. Fgf ligands, including Fgf2, and the Fgfr1 and Fgfr2 receptors are expressed by astrocytes and astrocytic stem cell lineages of the developing and adult CNS. Previous work has shown that the inactivation of Fgfr1 in radial glial progenitors of the developing cortex presents with an impairment in the postnatal maturation of parvalbumin positive interneurons (PV+). This interneuron defect is associated with increased locomotor hyperactivity. PV+ interneurons have an extensive postnatal maturation process which may be regulated by other cell types in the cortical environment, including glial cells expressing Fgfr1. We have characterized the expression of Fgfr1 in the developing cortex and hippocampus with the tgFgfr1-EGFP transgenic line (GENSAT). We demonstrate that there is an extensive expression of Fgfr1 in glial cells throughout postnatal development, at the time that interneuron maturation is occurring, and in DCX positive neuroblasts in the hippocampal dentate gyrus. We have further inactivated Fgfr1 in postnatal astrocytes by tamoxifen inducible Cre mediated recombination using the hGFAP-CreERT2 (GCE) transgene. We targeted astrocytes by postnatal injections of tamoxifen (P14-17, 60 mg/kg i.p.). Postnatal loss of Fgfr1 alone resulted in hyperactivity, and decreased anxiety on the elevated plus maze test. No differences in learning were observed on a modified one-day morris water maze test. Previous studies have shown that fgfr2 can have compensatory effects during the inactivation of fgfr1 (for example, in cerebellar development). We are currently investigating the effects of Fgfr1 and Fgfr2 double mutants mediated by tamoxifen inducible GCE recombination. We will determine whether the double mutants have a more extensive behavioral phenotype than Fgfr1 single mutants, and compare the effects of Fgfr1 single and Fgfr1/Fgfr2 double mutants upon PV neuron maturation, and postnatal hippocampal proliferation.

Disclosures: K.M. Smith: None. **L.B. Rubin:** None. **L. Choubey:** None. **J. Collette:** None. **R.R.H. Deegan:** None. **F.M. Vaccarino:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.04/B89

Topic: B.11. Glial Mechanisms

Support: La Fondation pour la Recherche Médicale

RIKEN SPR Fellowship to MA

Title: Dissecting the role of IP3-Receptor subtypes in hippocampal LTP

Authors: *M. W. SHERWOOD[1,2], M. ARIZONO[3], C. HISATSUNE[3], H. BANNAI[3,4], E. EBISUI[3], J. L. SHERWOOD[5], A. PANATIER[1,2], S. H. R. OLIET[1,2], K. MIKOSHIBA[3]; [1]NEUROCENTRE MAGENDIE, INSERM U862, Bordeaux Cedex, France; [2]Univ. de Bordeaux, Bordeaux, France; [3]Lab. for Developmental Neurobio., RIKEN Brain Sci. Inst., Wako-shi, Japan; [4]Div. of Biol. Sciences, Grad. Sch. of Sci., Nagoya Univ., Nagoya, Japan; [5]Dept. of Stem Cell and Regenerative Biol., Harvard Univ., Cambridge, MA

Abstract: Astrocytic Ca2+ signaling is required for LTP at the Hippocampal Schaffer Collateral to CA1 (SC-CA1) synapses. Controversy, however, remains as IP3 receptor (IP3R) type-2 knockout mice (IP3R-2KO), which are reportedly deficient in astrocytic Ca2+ signaling, exhibit normal LTP. It is possible that an astrocytic Ca2+ channel hitherto unknown is required for LTP and that some Ca2+ transients still prevail locally, at the level of the processes, in IP3R-2KO mice. In the current study, we address this hypothesis by visualizing sub-cellular Ca2+ dynamics within astrocytic processes, focusing on Ca2+ release through all IP3R subtypes (1,2,3) and their role in LTP. To image Ca2+ dynamics in astrocytic processes we used two-photon imaging of GCaMP3 expressed in cultured hippocampal slices. We also performed two-photon Ca2+ imaging in acute slices, in this case we loaded the Ca2+ indicator Fluo-4 via an astrocytic whole-cell patch-pipette. To evoke Ca2+ responses in astrocytes, we bath applied the group 1 metabotropic glutamate receptor agonist DHPG, or performed high frequency stimulation (HFS, 1sec at 100Hz) of the Schaffer Collaterals. In contrast to the premise of previous studies, we observed substantial astrocytic Ca2+ responses in slices prepared from IP3R-2KO mice. As the activation of astrocytic group 1 mGluRs is known to trigger Ca2+ release via IP3Rs, we decided to investigate the contribution of other IP3R subtypes. To this end we repeated the above experiments using hippocampal slices prepared from IP3R type-2/-3 double KO mice (IP3R-2/3KO) and used heparin, introduced via an astrocytic whole-cell patch-pipette, to inhibit all IP3R subtypes. We thus identified two new astrocytic Ca2+ channels, namely IP3R-1 and IP3R-3. Having identified two new functional Ca2+ channels in astrocytic processes we tested their involvement in LTP. In accordance with Ca2+ imaging data, 2xHFS-LTP was intact in slices prepared from IP3R-2KO, and IP3R-2/3KO mice. Finally, inhibiting all astrocytic IP3R subtypes with heparin inhibited 2xHFS-LTP. Our results show for the first time that IP3R-1 and IP3R-3 are functional Ca2+ channels within astrocytes, and that they could be required for LTP induction at hippocampal CA3-CA1 synapses.

Disclosures: **M.W. Sherwood:** None. **M. Arizono:** None. **C. Hisatsune:** None. **H. Bannai:** None. **E. Ebisui:** None. **J.L. Sherwood:** None. **A. Panatier:** None. **S.H.R. Oliet:** None. **K. Mikoshiba:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.05/B90

Topic: B.11. Glial Mechanisms

Support: Neuroinflammation Training Program

Max E. Binz Fellowship

Ronald Peter Griggs Fellowship

CIHR

NSERC

Title: TNFα suppresses drug induced synaptic plasticity and behavior

Authors: ***S. C. KONEFAL**[1], G. M. LEWITUS[1], K. AUGEREAU[2], S. CHIERZI[1], K. MURAI[1], D. STELLWAGEN[1];
[1]Ctr. for Res. in Neurosci., [2]McGill Univ., Montreal, QC, Canada

Abstract: Repeated administration of cocaine results in the development of behavioral sensitization in rodents, which is a progressive increase in locomotor response following repeated drug exposure. The nucleus accumbens (NAc) serves as a cross point between corticolimbic and motor regions, therefore playing an important role in generating motivated behaviors to natural rewards and drugs of abuse. Behavioral sensitization is accompanied by a decrease in excitatory synaptic strength in the NAc through an unknown mechanism, which slowly potentiates during withdrawal. Repeated exposure to cocaine also produces an enduring increase in dendritic spine density in the NAc. Here we show that repeated administration of cocaine activates microglia and induces TNFα expression in the NAc, which in turn depresses glutamatergic synaptic strength and limits the development of behavioral sensitization. Further, TNFα KO mice show an increased spine density and cocaine-induced spinogenesis in the NAc, suggesting a regulatory role for TNFα in dendritic spine formation or maintenance. TNFα is known to be produced by glia in the brain and glial cells are activated by a variety of drugs of abuse. Their contribution to the development of addictive behaviors is not well characterized. We utilized a Cre-loxP system to selectively delete TNFα from distinct subclasses of cells in the brain, CX3CR1-Cre mice for microglia and GFAP-Cre mice for astrocytes. Mice that lack microglia-derived TNFα show a significantly higher level of sensitization to cocaine.

Conversely, mice that lack astrocytic TNFα do not have elevated sensitization. We hypothesize that TNFα is part of an important adaptive response to chronic cocaine involving microglia. However, our results suggest that cocaine-induced activation of microglia and TNFα expression occurs only during a narrow window following cocaine exposure, and re-activating microglia could be therapeutic. Accordingly, we found that re-activation of microglia and induction of TNFα expression by a weak TLR4 agonist depresses synaptic strength in the NAc and acutely suppresses cocaine-induced sensitization without inducing sickness behavior. In conclusion, manipulating microglia and TNFα levels in the NAc is a potential avenue for regulating cocaine-induced plasticity and behavior.

Disclosures: **S.C. Konefal:** None. **G.M. Lewitus:** None. **K. Augereau:** None. **S. Chierzi:** None. **K. Murai:** None. **D. Stellwagen:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.06/B91

Topic: B.11. Glial Mechanisms

Support: CFI Grant 10435

NSERC 105112

OBI - POND

Title: The influence of immune and endocrine signaling on the neuroanatomy of the bed nucleus of the stria terminalis

Authors: *R. KHALID[1], J. A. FOSTER[1,2];
[1]Psychiatry and Behavioural Neurosci., McMaster Univ., Hamilton, ON, Canada; [2]St. Joseph's Healthcare, Brain Body Inst., Hamilton, ON, Canada

Abstract: The immune and endocrine system influence behaviour and contribute to the development of the central nervous system (CNS) in a sexually dimorphic manner. The bed nucleus of the stria terminalis (BST) is a highly sexually dimorphic brain region; in most mammalian species the male BST is larger than the female BST. Previously, our lab has shown a loss of this sexual dimorphism in mice lacking functional T cells. The present study investigates the mechanism by which T lymphocytes may be influencing sexual dimorphic development. Using immunohistochemistry and the microglial marker, anti-Iba1, microglia were examined in WT and mice lacking the β and δ chains of the T cell receptor (TCRβ-/-δ-/). Sex differences in brain volume of the BST are present early in postnatal life and microglial analysis of brain tissue from postnatal day 7 mice is ongoing. Serum levels of anti-Müllerian hormone (AMH), a

gonadal hormone implicated in development of the BST, will also be assessed in postnatal mice. Our preliminary results from adult mice showed significant sex and genotype effects between WT and TCRβ-/-δ-/- mice. In the dorsal BST, females showed a greater number of small microglia compared to males, and WT males had a greater number of small microglia compared to TCRβ-/-δ-/- males. In the ventral BST, the genotype effect in the male groups was reversed with WT males showing a reduced number of small microglia compared to TCRβ-/-δ-/- males. The influence of immune and endocrine signaling will be further explored during early development of TCRβ-/-δ-/- mice to illustrate the contribution of these systems on sexual dimorphic development of the brain. Acknowledgements: Funding support by the Ontario Brain Institute and the Canadian Foundation for Innovation.

Disclosures: R. Khalid: None. **J.A. Foster:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.07/B92

Topic: B.11. Glial Mechanisms

Support: AHA Grant RP0136

Title: Glial miRNA regulates Progesterone's neuroprotective function by altering the PGRMC1/KLF4 signaling

Authors: *T. NGUYEN, M. SINGH, C. SU;
Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Stroke has been reported as the fourth leading cause of death for Americans and it is a leading cause of adult disability. The risk of ischemic stroke increases significantly with aging. Gender appears to play a profound role, with the incidence being higher in women. A large body of studies has suggested that women in postmenopausal state are at greater risk of ischemic stroke and are likely to experience much more severe impacts. A considerable amount of research has supported that P4 is a potent neuroprotectant that may exert beneficial effects in various neurodegenerative disorders, including stroke. Our laboratory has reported that Brain-derived neurotrophic factor (BDNF), which has well-defined roles in synaptogenesis and neuronal survival is a critical mediator for P4 neuroprotective actions. We have also recently found that P4 enhances BDNF release from glia, but not from neurons, by acting via a novel membrane-associated progesterone receptor, Pgrmc1. We recently have identified a member of the Let-7 miRNA family that could potentially regulate PGRMC1 by suppressing its transcription. Furthermore, our data demonstrated an inverse association between Let-7 miRNA and Pgrmc1 expression levels in post-ischemic mouse hippocampus. This correlation was

reported in normal aging brain where the antagomir (synthetic inhibitor) of Let-7 miRNA significantly reduced infarct volume and improved neurological deficits in a rodent ischemic stroke mode. These lines of evidence have strongly supported our hypothesis that in normal aging or in the stroked brain, Let-7 miRNA negatively regulates Pgrmc1 gene expression, which disrupts P4-induced BDNF release from glia and ultimately leads to the attenuation of P4's positive effect on synaptogenesis and neuronal survival.

Disclosures: **T. Nguyen:** None. **M. Singh:** None. **C. Su:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.08/B93

Topic: B.11. Glial Mechanisms

Support: CIHR

Title: D-Serine promotes synapse maturation and axonal branch stabilization in the developing visual system of the Xenopus tadpole

Authors: ***M. VAN HORN**[1], L. POLLEGIONI[2], E. RUTHAZER[1];
[1]Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada; [2]Dept. of Biotech. and Mol. Sci., Univ. of Insubria, Varese, Italy

Abstract: The gliotransmitter D-serine is a co-agonist for N-methyl-D-aspartate receptors (NMDAR) and has been shown to modulate synaptic transmission and plasticity mediated by this receptor. Here we examined the role D-serine plays in shaping neuronal activity and axonal remodeling in the developing visual system of the Xenopus tadpole. We find acute D-serine (100 μM) wash-on acutely enhances NMDAR currents of optic tectal neurons, whereas degradation of D-serine by RgDAAO reduces NMDAR currents, indicating that endogenous D-serine is normally present below saturating levels. Using D-serine amperometric biosensors, we found that α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) activation results in an increase in D-serine release *in vivo*. To investigate the influence of D-serine availability on circuit maturation, we tested whether chronically elevating D-serine levels could influence the maturation of glutamatergic synapses. We find that tadpoles raised in D-serine (100 μM) for 2 days have higher frequencies of miniature excitatory postsynaptic AMPAR currents and higher retinotectal synaptic AMPA/NMDA ratios compared to control animals. Conversely, decreasing the amount of available D-serine, with a local injection of RgDAAO, results in a decrease in the amplitude and frequency of mEPSCs 24hrs after the injection. To examine the effects of D-serine on morphological development of retinotectal axons, images of EGFP expressing retinal axons were collected daily, over 4 days to assess growth and branch

elaboration and at shorter (10 min) intervals to assess branch stabilization. We find that increasing available D-serine results in the hyperstabilization of retinal axon branches, with axonal arbors becoming less complex compared to control axons over 4 days of treatment with D-serine. These findings are consistent with the hypothesis that D-serine enhancement of NMDAR currents promotes synaptic maturation and leads to stabilization of axonal branches. Taken together, these results suggest that D-serine levels are modulated by glutamatergic neurotransmission *in vivo* and promote the maturation of retinotectal synapses and axonal stabilization.

Disclosures: **M. Van Horn:** None. **L. Pollegioni:** None. **E. Ruthazer:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.09/B94

Topic: B.11. Glial Mechanisms

Support: Studentship Grant from the French Ministry of Higher Education and Research

Title: EphB3 receptors control synaptic NMDAR functions

Authors: ***V. C. LANGLAIS**, S. H. R. OLIET, A. PANATIER;
Neurocentre Magendie INSERM U862, Bordeaux Cedex, France

Abstract: Astrocytes are key partners of neurons and synapses. One of their main functions at CA3-CA1 hippocampal synapses is to regulate the activity of synaptic NMDA receptors (NMDARs) through the supply of the coagonist D-serine. Importantly, the detailed mechanism controlling the release of D-serine in the synaptic cleft is still unknown. In this work, we have investigated whether astrocytic EphB3 receptors play a role in controlling NMDAR coagonist availability and thus NMDAR functions. Using electrophysiological approaches on acute hippocampal slices of adult mice, we here show that exogenous stimulation of EphB3 receptors with clustered ephrinB3-Fc ligands leads to an increase of NMDAR activity at CA3-CA1 synapses. Importantly, this modulation is due to an increase of the coagonist-binding site occupancy. Furthermore, disrupting endogenous ephrinB3-EphB3 interaction induces an impairment of synaptic NMDAR activity and its associated long-term synaptic potentiation. Both are rescued by exogenous supply of D-serine. We are presently carrying out experiments, to assess the role of astrocytic versus neuronal EphB3 receptors, in this pathway. All together, our data reveals that EphB3 receptors regulate synaptic NMDAR functions through the control of the coagonist-binding site occupancy.

Disclosures: **V.C. Langlais:** None. **S.H.R. Oliet:** None. **A. Panatier:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.10/B95

Topic: B.11. Glial Mechanisms

Support: NIH COBRE Pilot Grant 1P20GM103653-01A1

NSF HBCU-UP Research Initiation Award HRD-1401026

Title: Astrocyte neuron interactions in synchronous bursting behavior

Authors: K. R. SANCHEZ, *M. TEMBURNI, M. HARRINGTON;
Biol., Delaware State Univ., Dover, DE

Abstract: Establishing functional neuronal networks during brain development requires synchronous oscillatory activity among neurons. However, the mechanisms of synchronization are not fully understood. Current models of neuronal synchronous activity assume that it is a process intrinsic to neurons. Evidence that glial cells particularly astrocytes modulate synchronous activity in networks of neurons is accumulating - for example during sleep, during prodromal oscillations preceding spreading depression, and the slow inward currents (SICs) resulting in synchronous activity in hippocampal neurons, thalamus and nucleus accumbens. Astrocytes participate in neuronal communication by releasing “gliotransmitters” like glutamate, ATP and D-serine. We hypothesize that astrocyte-neuron interactions are crucial for the development of synchronous activity seen in the developing vertebrate brain. We tested this hypothesis by establishing pure and mixed (astrocyte and neuronal) cultures from the developing chicken brain (optic tectum) and recording total neuronal activity using the multi-electrode array system, MED64. Pure neuronal cultures were obtained by treating cultures with the mitotic inhibitor 5-fluorodeoxyuridine (FUdR) which kills mitotically active astrocytes but spares post-mitotic neurons. Neurons were kept alive in the absence of astrocytes by supplementing the culture medium with 50% astrocyte conditioned medium. Mixed cultures of astrocytes and neurons show random spiking activity in one week and synchronous activity in two weeks whereas pure neuron only cultures show random spiking activity without synchronization even after two weeks - thus clearly establishing a role for astrocytes in the development of synchronous activity. To further confirm the involvement of astrocytes we have reintroduced astrocytes into the randomly spiking pure neuronal cultures after synchronous activity was observed in the control mixed cultures. We observed an immediate increase in spiking activity which synchronized within a week of reintroduction of astrocytes into the FUdR treated pure neuronal cultures. To further dissect the molecular pathways involved we are targeting GPCR pathways within astrocytes that mediate intracellular Calcium release. Activation of these G-protein coupled receptors by their respective neurotransmitters mobilizes intracellular calcium release leading to exocytosis of either glutamate or ATP. We are expressing dominant negative

peptides designed to disrupt downstream signaling pathways of these receptors and thereby calcium mobilization and exocytosis of gliotransmitters in chick embryo astrocytes.

Disclosures: K.R. Sanchez: None. **M. Temburni:** None. **M. Harrington:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.11/B96

Topic: B.11. Glial Mechanisms

Support: NSF Grant 924372

Title: Measurement of H+ fluxes from cultured rat cortical astrocytes using self-referencing H+-selective microelectrodes

Authors: *J.-I. CHOI[1,2], C. GOEKE[4,5], M. GUIZZETTI[4,5], R. P. MALCHOW[2,3];
[1]Univ. of Illinois At Chicago, Chicago, IL; [2]Biol. Sci., [3]Ophthalmology & Visual Sci., Univ. of Illinois at Chicago, Chicago, IL; [4]Dept. of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [5]VA Portland Hlth. Care Syst., Portland, OR

Abstract: Glial cells are believed to play a key role in regulating extracellular levels of glutamate in the nervous system, transporting extracellular glutamate into the cytoplasm of the glial cells. This transport process is often associated with changes in extracellular levels of H+, which can by itself have a potent modulatory effect on neuronal excitability and synaptic transmitter release. In the present study, we have used self-referencing H+-selective microelectrodes to examine standing levels of extracellular H+ from quiescent cortical astrocytes cultured from rats, and have also examined changes in the level of extracellular H+ that occur upon addition of the neurotransmitter glutamate. Cultured astrocytes in a bicarbonate-based saline solution exhibit a standing acidic flux. When the normal 24 mM bicarbonate in the solution is replaced with 1 mM HEPES, a standing flux remains but is smaller in magnitude. The standing flux observed in the 1 mM HEPES condition remained when all of the extracellular sodium was replaced with choline. Application of glutamate induced a transient extracellular alkalinization, consistent with its transport into the glial cells. These results are the first to show extracellular H+ levels adjacent to quiescent glial cells and suggest the possibility that changes in extracellular H+ by glial cells may play a role in modulation of activity within the nervous system.

Disclosures: J. Choi: None. **C. Goeke:** None. **M. Guizzetti:** None. **R.P. Malchow:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.12/B97

Topic: B.11. Glial Mechanisms

Title: Directed differentiation of human induced pluripotent stem cells to astrocytes for the study of spinal muscular atrophy

Authors: *S. LOH[1,2], L. W. STANTON[1,2];
[1]Stem Cell and Regenerative Biol., Genome Inst. Singapore, Singapore, Singapore; [2]Sch. of Biol. Sci., Nanyang Technological Univ., Singapore, Singapore

Abstract: Spinal Muscular Atrophy (SMA) is a neuromuscular childhood disease. SMA arises due to the mutation of *SMN1*. Motor Neurons (MNs) are particularly sensitive to the low levels of functional SMN protein and they selectively degenerate. In SMA mice models, overexpression of the SMN protein in MNs alone did not confer extended survival or increase muscle mass. However, when SMA protein levels were increased in both MNs and astrocytes, SMA mice displayed improved survivability and muscle mass, indicating the importance of astrocytes in SMA pathology. To study the roles of astrocytes in SMA pathology in human genetic background, we established an efficient method of generating SMA and WT astrocytes from induced pluripotent stem cells (iPSCs). We are performing studies such as glutamate uptake tests and calcium imaging on the iPSCs-derived astrocytes to uncover functional differences between WT and SMA conditions. We are also examining the levels of intracellular reactive oxygen species to determine the presence of cellular oxidative stress in iPSCs-derived astrocytes, which is an important indicator of astrocytes pathology. We hypothesize that iPSCs-derived SMA astrocytes will exhibit functional abnormalities and have the propensity of inducing cell death of the surrounding MNs in SMA conditions.

Disclosures: S. Loh: None. **L.W. Stanton:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.13/B98

Topic: B.11. Glial Mechanisms

Support: National Natural Science Foundation of China (No. 81072242 and No. 81272576)

Title: Determining the role of TREM-2 in cerebral ischemic reperfusion injury

Authors: *Y. TANG, X. RONG, P. XU, Y. XU, R. WU;
Sun Yat-Sen Mem. Hospital, Sun Yat-Sen Univ., Guangdong, China

Abstract: Background: Ischemic stroke remains a public health burden across the globe. Although thrombolysis is the class I recommendation for treatment, it may lead to ischemic reperfusion injury. Immunoinflammatory response is a profound pathogenic mechanism existing in the whole process of ischemic reperfusion. TREM2 (Triggering receptor expressed on myeloid cells - 2), belonging to the immunoglobulin and lectin-like superfamily, can facilitate phagocytosis and inhibit pro-inflammation. However, its role in the cerebral ischemic reperfusion injury is still unclear. **Objectives:** To estimate the role of TREM2 in cerebral ischemic reperfusion injury. **Materials and methods:** *In vitro* study, we established an oxygen-glucose deprivation and reoxygenation (OGDR) model with primary microglia to detect the intracellular mRNA and protein expression of TREM2. By using TREM2 siRNA Oligonucleotides, we further compared the secretion of inflammatory cytokines such as TNF-α,IL-1β,iNOS and IL-10 in microglia among different TREM2 levels. In addition, using the co-culture model of primary hippocampal neurons and primary microglia, we evaluated the effect of microglia with different TREM2 level on neuron apoptosis. *In vivo* study, male C57BL/6J mice were used to establish middle cerebral artery occlusion (MCAO) model. We firstly determined the location and expression of TREM2 as well as the expression of a series of inflammatory cytokines. After injection of TREMs siRNA to the right latreal ventricle of the mice, the change of infarct area and the number of apoptotic neurons in peri-infract area were detected by TTC staining and double immunofluorescent staining. **Results:** We found that the expressions of TREM2 in microglia after OGDR increased with a peak at 24 hours and then gradually decreased. So did the trend of the TREM2 expressions in peri-infarct area in MCAO mice. Through TREM2 knockdown, the IL-1β secretion increased at 6 hours and TNF-a, IL-1β, iNOS expression increased at 12 hours,while IL-10 decreased both at the two time points. By overexpressing TREM2, IL-1β as well as TNF-a, IL-1β, iNOS and IL-10 showed a contrary trend. Being co-cultured with OGDR microglia, more primary hippocampal neurons suffered from apoptosis, and the injury could be alleviated by overexpressing TREM2. In MCAO model, TREM2 mostly expressed in microglia which located in peri-infarct area. Lower expression of TREM2 leaded to a higher level of inflammatory cytokines and poor neurological outcomes. **Conclusions:** TREM2 could inhibit the expression of pro-inflammatory cytokines and neuronal apoptosis, which is good for tissue repair and neurological recovery.

Disclosures: **Y. Tang:** Other; This work was supported by National Natural Science Foundation of China (No. 81072242 and No. 81272576). **X. Rong:** None. **P. Xu:** None. **Y. Xu:** None. **R. Wu:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.14/B99

Topic: B.11. Glial Mechanisms

Support: King Abdullah University of Science and Technology Competitive Research Grant (CRG) Program

The EPFL Blue Brain Project Fund

Title: Model of electro-metabolic coupling of the neuro-glia-vasculature in the cerebral cortex explores role of the glycogen shunt

Authors: *J. S. COGGAN[1], D. KELLER[1], J. G. KING[1], C. CALI[2], H. LEHVASLAIHO[2], F. SCHÜRMANN[1], H. MARKRAM[1], P. J. MAGISTRETTI[2];
[1]Blue Brain Project / EPFL, Geneva, Switzerland; [2]King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia

Abstract: The energy requirements of signaling in the cerebral cortex are met by a variety of metabolic cascades that take place within neurons and glia and are supplied by the local microvasculature. The shuttle of lactate from glia to neurons (ANLS) constitutes a critical energy baton between the two cell types that sustains the energetic requirements of synaptic transmission. This energy relay is also critical for the maintenance of the transmembrane concentration balance of the neurotransmitter glutamate. Of particular interest is the relative sourcing of lactate from either glycogen mobilization or glycolysis. Glycogen is located within the glia and is thought to be a storage center for glucose and the lactate that is eventually transported to the energy-requiring neurons. The metabolic cost of maintaining a glycogen shunt for the storage and retrieval of glucose for lactate production is greater than that of glycolysis, thus begging the question of the role of this alternative pathway. The neurological and psychiatric deficits presented by patients with glycogen metabolism disorders also point to a significant constitutive function of the glycogen shunt in the brain. Using a computational model of the neuro-glia-vasculature (NGV) within a cortical column containing synthetic astrocytes, we explored the metabolic behavior of the NGV during various activity regimens, focusing on the contribution of the glycogen shunt to metabolic patterns arising from frequency-dependent cortical activity. We investigated the relative timing of ANLS development versus glycogen shunt activity to determine the contribution of glycogen to the oxygen to glucose index (OGI). We further examined neurotransmitter-mediated regulation of glycogen formation by norepinephrine (NE) and vasoactive intestinal peptide (VIP) and asked how they impact the activation of cAMP-dependent kinases, the regulation of glycogen and OGI under conditions of normal and enhanced synaptic transmission.

Disclosures: **J.S. Coggan:** None. **D. Keller:** None. **J.G. King:** None. **C. Cali:** None. **H. Lehvaslaiho:** None. **F. Schürmann:** None. **H. Markram:** None. **P.J. Magistretti:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.15/B100

Topic: B.11. Glial Mechanisms

Support: NIH R01 HL088090

VA Merit Review

Title: Astrocytic lesions that spare neurons in the nucleus tractus solitarii interfere with cardiorespiratory control

Authors: G. B. RICHERSON[1], D. N. DRAGON[2], S. JONES[2], Y. WU[2], *W. T. TALMAN[2,3]; [1]Neurol., [2]Carver Col. of Medicine, Univ. Iowa, Iowa City, IA; [3]Neurol., Dept. of Veterans Affairs Med. Ctr., Iowa City, IA

Abstract: Conjugates of saporin (SAP) have been widely used to target specific neurons while leaving other neurons undisturbed. We found that killing catecholamine neurons bilaterally in the nucleus tractus solitarii (NTS) by injection of the SAP conjugate containing an antibody to dopamine-β-hydroxylase (antiDBH-SAP) spared non-catecholamine neurons but led to attenuation of baroreceptor reflexes, lability of arterial pressure, and, in some animals, sudden death. In contrast, selective targeting of catecholamine neurons with 6-hydroxydopamine produced no such cardiovascular events. We hypothesized that SAP conjugates may target non-neuronal cells in the NTS. Indeed, we found that local astrocytes were killed by the conjugates as well as by unconjugated SAP itself. SAP injections into the NTS led to death of astrocytes that expressed glial fibrillary acidic protein (GFAP) but did not affect neuronal structural markers and neuronal biosynthetic enzymes. Our recent studies further suggest that local neurons are physiologically intact. Nonetheless, SAP injections into the NTS significantly reduced cardiovascular responses elicited by glutamate agonists injected into the NTS, and bilateral injections of SAP into the NTS led to attenuation of cardiovascular reflexes whose pathways pass through the NTS, lability of arterial pressure, damage to cardiac myocytes and sudden death resulting from asystole. When asystole and death followed SAP treatment the fatal arrhythmia followed progressive bradycardia. In that treated animals demonstrate altered ventilatory function, we conjecture that it is altered ventilation that leads to cardiac compromise and death.

Disclosures: G.B. Richerson: None. **D.N. Dragon:** None. **S. Jones:** None. **Y. Wu:** None. **W.T. Talman:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.16/B101

Topic: B.11. Glial Mechanisms

Support: NIH Grant 5 T32 NS 58280-8

NIH Grant NS060677

Title: Cellular contributions of gap junctions to gamma oscillations in the hippocampus

Authors: *A. D. JOHNSTON, B. S. KHAKH;
Physiol., UCLA, Los Angeles, CA

Abstract: Gap junctions, formed by connexin proteins, function as conduits between the intracellular milieu of many cell types. These pores have been difficult to study due to their distal locations at junctions and the lack of selective pharmacological tools. In neurons, they form electrical synapses which are important for synchronizing neural circuits. For example, it has been previously described that removal of neuronal connexin36 diminishes gamma oscillations in the CA3 region of the mouse hippocampus. These oscillations, ranging from 20-100 Hz are thought to underlie attention, learning, and working memory. Gap junctions are also found in abundance in other cell types of the brain, including astrocytes that exist in numbers at least equal to neurons and form close spatial interactions with pyramidal neurons and interneurons. Astrocyte gap junctions play important developmental roles and facilitate intercellular coupling and regulation of synaptic transmission. However, their contributions to circuit level functions such as gamma oscillations are not well described. Additionally, unopposed connexin and pannexin pores, known as functional hemichannels, release neuromodulators such as ATP and glutamate which have also been shown to modulate neuronal activity. Using targeted genetic and pharmacological approaches, we investigated the contribution of gap junctions and hemichannels in both neurons and astrocytes to network oscillations in the hippocampus. Using a broad pharmacological approach, we have confirmed that functional gap junctions are required for the maintenance of gamma oscillations and are developing genetic approaches to specifically disrupt junctional coupling in astrocytes. Additionally, we have found that, while functional hemichannels exist in hippocampal astrocytes, the function of pannexin hemichannels does not seem to be necessary for gamma oscillations. Overall, we will report new data on how gap junction proteins within astrocytes affect gamma oscillations in the hippocampus.

Disclosures: A.D. Johnston: None. **B.S. Khakh:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.17/B102

Topic: B.11. Glial Mechanisms

Title: Pyruvate carboxylase rate determined in awake rats using [2-13C]glucose

Authors: *L. F. MCNAIR[1], G. F. MASON[2], H. S. WAAGEPETERSEN[1], K. L. BEHAR[3]; [1]Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Kobenhavn O, Denmark; [2][1] Dept. of Diagnos. Radiology, Magnetic Resonance Res. Ctr. [2] Dept. of Psychiatry, [3]Dept. of Psychiatry, Magnetic Resonance Res. Ctr., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Our knowledge about the brain is steadily increasing; still, essential metabolic functions of brain cells have not been fully characterized. The astrocyte, which is a glial cell abundant in the mammalian brain, has an important role in anaplerosis by housing pyruvate carboxylase (PC), the enzyme needed for *de novo* synthesis of glutamate. The PC rate has been measured *in vivo* in single cortical samples of human and rodent brain, but not yet regionally. The aim of this study was to apply MRS with [2-^{13}C]glucose to measure the PC rate in several brain regions of the awake rat. 57 freely moving young adult male Sprague-Dawley rats (210±10g, mean±SD) were infused with either [1-^{13}C] or [2-^{13}C]glucose. 8, 15, 30, 60 or 120 min after start of glucose infusion rats were euthanized by Focused Beam Microwave Irradiation (n = 5-7 per time point), blood was collected and the brain removed and dissected into 6 regions: cortex, cerebellum, hippocampus, striatum, thalamus, and hypothalamus. Extracted brain and plasma samples were analyzed by proton-observed carbon-edited NMR spectroscopy to obtain time courses of ^{13}C-enrichments at different carbon positions of glutamate, glutamine, and GABA, as well as total metabolite concentrations. These data were combined and fitted in a three-compartment model (i.e. astrocyte, GABAergic and glutamatergic neuron) using CWave (Graeme F. Mason, Yale University), to derive the metabolic fluxes. Analysis of cortex extracts from [2-^{13}C]glucose infusions revealed that by 120min of infusion, enrichments at glutamine C3, glutamate C3 and GABA C3, resulting predominantly from PC activity, reached 6-8%. Glutamate C4, glutamine C4 and GABA C2 from [1-^{13}C]glucose infusions reached 30-40% at 120min, and formed the primary basis to estimate tricarboxylic acid cycle flux (V_{TCA}) and the glutamate-glutamine cycle (V_{cycle}) flux, happening in and between both neurons and astrocytes. Metabolic rates from initial modeling of cortex data were V_{cycle}=0.73, V_{TCA}=1.72, and V_{PC}=0.38umol/min/g. Further data analysis is needed to finalize metabolic rates for all brain regions.

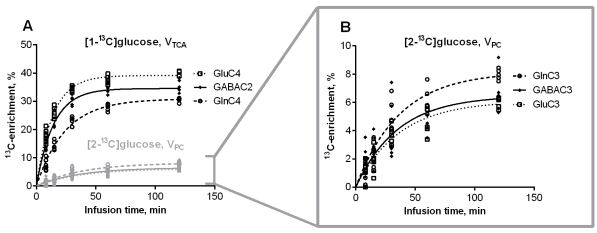


Fig. ^{13}C-enrichment time courses for rat cortex illustrating that pyruvate carboxylase rate (V_{PC}) is lower than the rate of tricarboxylic acid cycle (V_{TCA}). (A) Black symbols/lines, showing glutamate C4 (GluC4), GABA C2, and glutamine C4 (GlnC4) enrichments from [1-^{13}C]glucose infused rats (*n = 27*) are significantly higher than glutamate C3 (GluC3), glutamine C3 (GlnC3), and GABAC3 from [2-^{13}C]glucose infused rats (*n = 30*) shown in gray. **(B)** An amplification of the gray time courses for glutamate, glutamine and GABA C3 shown in (A) enabling better visual separation of these. Metabolic rates from initial modelling of data: V_{cycle}=0.73, V_{TCA}=1.72, V_{PC}=0.38 umol/min/g. *n* for each time point is 5-7 rats.

Disclosures: **L.F. McNair:** None. **G.F. Mason:** None. **H.S. Waagepetersen:** None. **K.L. Behar:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.18/B103

Topic: B.11. Glial Mechanisms

Title: Astrogliosis: changes in astrocyte KIR expression and function in Alzheimer's

Authors: ***L. M. OSBORN**[1], L. KOOIJMAN[1], W. KAMPHUIS[2], W. J. WADMAN[1], E. M. HOL[3];
[1]Univ. of Amsterdam, Amsterdam, Netherlands; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [3]Dept. of Translational Neurosci., Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: Alzheimer's disease (AD) is the main cause of dementia in the elderly and begins with a subtle decline in episodic memory followed by a more general decline in overall cognitive abilities. Though the exact trigger for this cascade of events remains unknown the presence of the misfolded amyloid-beta (Aß) protein triggers reactive gliosis, a prominent neuropathological feature in the brains of AD patients. lt is likely that the physiological function of astrocytes is affected in AD, resulting in altered synaptic physiology and having consequences for the stability of microcircuits within key brain regions. We analyzed microarray data from an aged mouse model of Alzheimer's (APPswe/PS1dE9) and found crucial changes in astrocyte-specific genes associated with ion homeostasis including a global down-regulation of glutamate (GLT-1 and GLAST) and GABA (GAT-1) transporters, Na/K-ATPase (AMOG), and outward (TWIK-1 and TREK2) and inward potassium rectifiers (Kir4.1 and Kir5.1). Further immunohistochemical analysis of KIR expression surprisingly revealed localized increases of channel expression in astrocytes directly surrounding amyloid plaque deposits. By focusing on potassium currents in the membrane using patch-clamp and field potential recordings we were characterized the electrophysiological properties of astrocytes during several stages of astrogliosis. We investigated the KIR conductance in astrocytes surrounding plaques as well as those further from plaque deposition and were able to show that reactive astrocytes surrounding plaques have a higher potassium conductance when compared to those further from plaque pathology. Additionally, we investigated local synaptic activity by using the astrocytes as a field potential sensor (aEPSPs). We also determined the potassium current in the astrocytes as a direct consequence of stimulation of the surrounding neuronal network. The latter observations were compared to currents induced by fast local pressure application of a high potassium solution. Our preliminary results suggest that functional changes in reactive astrocytes disrupt potassium homeostasis with potential consequences for normal neuronal and synaptic function. We

hypothesize that astrogliosis is an important player in the development of dementia. If astrocytes have a modulatory role in normal neuronal communication, reactive astrocytes are likely to interfere with synaptic efficacy and plasticity.

Disclosures: L.M. Osborn: None. **L. Kooijman:** None. **W. Kamphuis:** None. **W.J. Wadman:** None. **E.M. Hol:** None.

Poster

297. Astrocyte-Neuron Interactions II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 297.19/B104

Topic: B.11. Glial Mechanisms

Support: Helmholtz Virtual institute "RNA dysmetabolism in Amytrophic Lateral Sclerosis and Frontotemporal Dementia"

Title: Specific expression and function of the metabolic master regulator PGC-1α in the brain

Authors: *H. BAYER[1], E. BARTH[1], T. LUCAS[2], I. MERDIAN[1], P. WEYDT[1], A. WITTING[1]; [1]Exptl. Neurol., [2]Dept. of gene therapy, Ulm Univ., Ulm, Germany

Abstract: Peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-α (PGC-1α) is a transcriptional coactivator involved in metabolic responses. We recently reported specific PGC-1α isoforms which are under the control of a brain-specific promoter1. The role of these brain-specific PGC-1α isoforms is currently unknown. There is evidence that PGC-1α contributes to the pathogenesis of neurodegenerative disease spectrum disorders. We investigated the expression of canonical PGC-1α and its brain-specific isoforms in different brain areas and primary cells of the brain. Further we focused on their cell-specific function. We found that brain-specific PGC-1α isoforms are mainly expressed in neurons. Furthermore PGC-1α expression is stimulated by lactate selectively in neurons. This increased PGC-1α expression induced only selected PGC-1α target genes suggesting a specific role of PGC-1α in the brain. Co-transfection of neuro2a cells with the canonical or brain-specific PGC-1α isoforms and luciferase-constructs containing promoters of known PGC-1α target genes showed diverging activities of the different brain-specific PGC-1α isoforms compared to the canonical form. Also a microarray analysis of striatum of PGC-1α knock-out animals suggests a divergent functional role of PGC-1α in the brain in contrast to other peripheral tissues. We identified and confirmed via qPCR BDNF, SOD3 and PDK4 as down-regulated in PGC-1α knock-out neurons. The expression of these genes is increased by lactate stimulation. Our results suggest that PGC-1α might have a specific role in the brain energy metabolism. References 1. Soyal SM, Felder TK, Auer S, et al. A greatly extended PPARGC1A genomic locus encodes several new brain-specific isoforms and influences Huntington disease age-of-onset. Hum Mol Genet. 2012.

Acknowledgements: The work is funded by the Helmholtz Virtual institute “RNA dysmetabolism in Amytrophic Lateral Sclerosis and Frontotemporal Dementia” (to AW and PW).

Disclosures: **H. Bayer:** None. **E. Barth:** None. **T. Lucas:** None. **I. Merdian:** None. **P. Weydt:** None. **A. Witting:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.01/B105

Topic: B.11. Glial Mechanisms

Support: JSPS KAKENHI Grant Number 26460094

JSPS KAKENHI Grant Number 26117504

Title: Status epilepticus increases microglial engulfment of newborn cells

Authors: ***C. LUO**, K. RYUTA, Y. IKEGAYA;
The Univ. of Tokyo., Tokyo, Japan

Abstract: Microglia, the brain-resident immune cells, contribute to the homeostasis of neural circuits via phagocytosis of dead cells, cell debris, and immature synapses. It has been suggested that microglia regulate adult neurogenesis in the dentate gyrus via phagocytosis of dead neural progenitor cells (NPCs). Newborn NPCs increases in the brain of patients with temporal lobe epilepsy and their animal models; however, whether and how microglia are involved in the aberrant proliferation and survival of NPCs after seizures remain unclear. Here, we show that the engulfment of newborn cells by microglia is facilitated after status epilepticus in mice, which regulates the density of newborn cells in the dentate gyrus. We induced status epilepticus (SE) by administering kainic acid in both wild-type and CX3CR1-GFP mice, in which microglia are labeled by GFP. Then, we labeled post-SE proliferating newborn cells with 5-ethynyl-2'-deoxyuridine (EdU) and immunohistochemically assessed the interaction between EdU-labeled newborn cells and microglia. We found that the phagocytosis rate of post-SE born cells by activated microglia increased in SE mice and most post-SE born cells were ablated in one week. Thus, our findings suggest that microglia may contribute to the homeostasis of neural circuits by engulfing excess newborn cells after seizures.

Disclosures: **C. Luo:** None. **K. Ryuta:** None. **Y. Ikegaya:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.02/B106

Topic: B.11. Glial Mechanisms

Support: CONACyT Grant 151261

CONACyT Grant 181323

Title: Microglial modulation affect respiratory rhythm generation and autoresuscitation

Authors: *J. LOREA[1], T. MORALES[2], F. PEÑA[2];
[1]neurobiología del desarrollo y neurofisiología, [2]Univ. Nacional Autónoma De México (UNAM), Queretaro, Mexico

Abstract: Pro-inflammatory conditions disturb respiratory function which in turn has been associated with the induction of apneas and Sudden Infant Death Syndrome. Several proinflammatory mediators inhibit breathing when applied peripherally or directly into the central nervous system (CNS). Considering that peripheral inflammation can activate microglia in the CNS and that this cell type can directly release all proinflammatory mediators that regulate breathing, it is likely that microglial activity can regulate breathing generation. It might even do so more prominently in hypoxia, since microglia is highly sensitive to hypoxia and peripheral proinflammatory conditions affect gasping generation and autoresuscitation. Here, we tested whether or not microglial activation with lipopolysaccharide (LPS) or inhibition with minocycline (MIN) affected respiratory rhythm generation (in normoxia and hypoxia) both *in vivo* and *in vitro*. By measuring breathing by plethysmographic means as well as the activity of the respiratory rhythm generator (the preBötzinger complex), we found that both LPS and MIN, applied intracisternally *in vivo* or in the recording bath *in vitro*, affect the generation of the respiratory rhythms both in normoxia and hypoxia and, in the case of LPS, affected the ability of the animals to autoresuscitate from hypoxic conditions. Moreover, we found that the inhibitory effect of MIN on the respiratory rhythm generator *in vitro* were reproduced by the microglial toxin l-leucine methyl ester, which dramatically reduced microglia in the brainstem slice preparation without affecting neuronal activity. In conclusion, our data show that microglial modulation alters respiratory rhythms generation and autoresuscitation.

Disclosures: J. Lorea: None. **T. Morales:** None. **F. Peña:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.03/B107

Topic: B.11. Glial Mechanisms

Support: CIHR

HSFC

FRSQ

Title: Microglia rapidly adopt a filopodia-rich phenotype upon oxygen depletion by sensing tissue acidosis

Authors: *L.-P. BERNIER, L. DISSING-OLESEN, J. K. HEFENDEHL, J. M. LEDUE, B. A. MACVICAR;
Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Microglia are highly motile cells that play a pivotal role in monitoring brain homeostasis by constantly probing the environment and responding to extracellular cues. They are involved in long-term stroke recovery, however the acute responses of microglia to the metabolic stresses of ischemia remain unclear. Here, we used two-photon imaging *in vivo* and in acute brain slices to monitor the initial effect of anoxia on the morphological phenotype and dynamic properties of microglia. The highly ramified morphology of resting microglia is rapidly transformed during oxygen depletion with extension of fine actin-dependent filopodia followed by retraction of microtubule-dependent ramifications. This rapidly reversible switch in morphology drives significant changes in microglial sensing behavior, affecting microglial cells capacity to respond to tissue damage. Our observations indicate that increased intracellular cyclic AMP is a key trigger of both filopodia extension and retraction of ramifications. During short anoxia insults, this phenotypic switch is induced by the accompanying acidic shift in the extracellular environment. This pH drop causes microglia to adopt a filopodia-rich phenotype through an increase in cyclic AMP induced by activation of the Gs-coupled proton-sensing receptor TDAG8. Characterizing the highly specialized sensing structures of microglia and defining the molecular cues responsible for the functional switch of microglial behaviour observed upon oxygen depletion will likely provide promising targets for stroke treatment.

Disclosures: L. Bernier: None. **L. Dissing-Olesen:** None. **J.K. Hefendehl:** None. **J.M. Ledue:** None. **B.A. MacVicar:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.04/B108

Topic: B.11. Glial Mechanisms

Support: MOST 103-2320-B-010 -015

CI -104 -10

Title: Selective inhibition of sEH phosphatase function attenuates oxygen-glucose deprivation/reperfusion-induced microglial activation

Authors: *H.-T. LEE;
No 155, Section 2, Li-Nong Street, Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Stroke, also known as cerebrovascular accident, is a condition caused by the interruption of blood supply to the brain, resulting to neuroinflammation due to lack of oxygen and nutrients. Our study used oxygen-glucose deprivation followed by reperfusion (OGD/R) 24 hours to mimic early stage of ischemia- reperfusion phase. Microglial cells are the resident macrophages in health brain, they constantly monitor their environment for signals from the CNS by moving their cellular processes. Upon encountering insults such as ischemic-hypoxia attack, microglia undergo transformation in morphology, proliferation, and becoming immune effector cells by releasing pro-inflammatory molecules and increasing expression of immunomodulatory surface antigens. However, it is now well-documented that microglia can function not only in pro-inflammatory but also neuroprotective fashion via growth factors release and immune response modulation which both can promote regeneration. Here, we present one novel candidate for such function: soluble epoxide hydrolase (sEH) whose expression is elevated in various brain injury models. sEH, is a bi-functional pro-inflammatory enzyme, with wide distribution in various tissues. Acute stroke leads to neuroinflammation which could trigger the production and release of arachidonic acid which is subsequently converted into epoxyeicosatrienoic acids (EETs). EETs have been shown to exert anti-apoptotic and anti-inflammatory effects. The C-terminal epoxide hydrolase function of sEH, is responsible for the metabolic conversion of EETs into dihydroxyeicosatrienoic acids (DHET). The N-terminal lipid phosphatase (PT) function of sEH, is thought to act on lipophilic compounds, but recent study has shown that it may also directly act on phosphorylated proteins, resulting complex pathway regulation. In our study, we found that mouse microglial cell, BV2 after OGD/R showed elevated sEH expression and morphological changes indicating activation. We then used sEH selective functional inhibitors, AFC and AUDA to target N- and C- terminus, respectively to delineate which domain is responsible for microglia activation. Our results indicated that inhibition of sEH phosphatase function leads to less morphological activation.

Disclosures: H. Lee: None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.05/B109

Topic: B.11. Glial Mechanisms

Support: National Natural Science Foundation of China [31171029]

Title: Annexin-1 promotes microglial activation and migration during oxygen-glucose deprivation/reperfusion via formyl peptide receptors

Authors: *L. LIU, Y. GAO, S. LIU, J. SHI;
Dept. of Neurobio., Tongji Med. College, HUST, Hubei, China

Abstract: Annexin-1 (ANXA1) has been shown to have neuroprotective effects in the brain, and microglia play significant roles during CNS injury, yet the underlying mechanisms remain unclear. In this study, we sought to determine if ANXA1 regulates the microglial response to oxygen-glucose deprivation/reperfusion (OGD/R) treatment in rat hippocampal slices, BV-2 cells (a microglial-like cell line) and primary microglia. Using immunofluorescence imaging, we determined that expression of ANXA1 and formyl peptide receptors (FPRs), which are targeted by ANXA1, were increased after OGD/R treatment in hippocampal slices. Correspondingly, microglial activation in the CA1 region of the hippocampus was enhanced by OGD/R treatment. These effects were reversed by the FPR antagonist brother of CDO-1 (Boc1). In trans-well and scratch wound migration assays, we found that Ac2-26(the N-terminal peptide of ANXA1) treatment during OGD enhanced BV-2 migration, whereas Boc1 treatment during OGD/R inhibited migration. In primary microglia, we proved that AC2-26 could polarize microglia to an anti-inflammatory M2 phenotype to protect neurons from ischemia-like injury. Taken together, our findings indicate that ANXA1 promotes microglial activation and migration during OGD/R via FPRs to protect neurons.

Disclosures: L. Liu: None. **Y. Gao:** None. **S. Liu:** None. **J. Shi:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.06/B110

Topic: B.11. Glial Mechanisms

Title: The induction of hippocampal long-term potentiation increases the motility of microglial processes and their engagement with dendritic spines

Authors: *T. PFEIFFER, E. AVIGNONE, V. NAGERL;
CNRS UMR 5297, Univ. of Bordeaux, Interdisciplinary Inst. For Neurosci., Bordeaux, France

Abstract: In the healthy brain microglia continuously scan their surroundings by rapidly extending and retracting their highly ramified processes. During this scanning activity microglia establish transient contacts with synapses in an activity-dependent way. Microglia can sense

changes in neuronal activity and their processes are steered towards active neurons, which may be mediated through the activation of neuronal NMDARs. Furthermore, recent work has implicated microglia in synapse remodeling during development and experience-dependent plasticity, giving rise to the idea that microglia might play a role in synaptic plasticity. However, very little is known about how microglia actually associate with synapses during basal synaptic transmission, and whether microglia can sense and respond to the induction of synaptic plasticity. Here, we investigated these questions using a combination of two-photon time-lapse imaging and electrophysiological recordings in acute hippocampal brain slices from transgenic mice, where microglia and neurons are fluorescently labeled. We analyzed the scanning motility of microglia and their physical interactions with dendritic spines of CA1 pyramidal neurons before and after the induction of hippocampal LTP using high-frequency electrical stimulation (HFS). Our analysis reveals that at any point in time only a very small fraction of dendritic spines were contacted by a microglial process. However, due to their high motility this fraction grew substantially over time. Contacts between microglial processes and dendritic spines were brief during basal synaptic transmission. After LTP induction microglial scanning motility was enhanced, while contact duration was prolonged and their number reduced. The application of the NMDAR antagonist APV prevented the HFS-induced changes in microglial scanning behavior and microglia-spine interactions. The elevated microglial motility and microglia-synapse contact stability during LTP corroborate the idea that microglia contribute to activity-dependent remodeling of synapses in the healthy mature brain. Recently, we have also used super-resolution STED microscopy to investigate the dynamic interactions between microglial processes and dendritic spines at the nanoscale in acute brain slices.

Disclosures: **T. Pfeiffer:** None. **E. Avignone:** None. **V. Nagerl:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.07/B111

Topic: B.11. Glial Mechanisms

Title: Erythropoietin attenuated the microglial cytotoxicity

Authors: *T. TAMURA[1], M. AOYAMA[2], H. KAKITA[3,4], S. UKAI[4], K. SOBUE[5], K. ASAI[4]; [2]Mol. and Cell. Pathobiology and Therapeut., [1]Nagoya City Univ., Nagoya, Japan; [3]Neonatology and Pediatrics, [4]Mol. Neurobio., [5]Anesthesiol. and Intensive Care Med., Nagoya City Univ. Grad. Sch. of Med. Sci., Nagoya, Japan

Abstract: Recently, it has been demonstrated that erythropoietin (EPO), a hematopoietic hormone, has a neuroprotective effect and EPO receptor (EPOR) is expressed in the central nervous system (CNS). In our previous study, brain immune cells microglia had higher EPOR

expressions than astrocytes and neurons. In this study, we investigated whether EPO could attenuate the cytotoxic activity of microglia stimulated by lipopolysaccharide(LPS). *In vitro*, we assessed the effect of EPO using microglial cell line BV-2 activated by LPS. The gene expressions of cytokines were analyzed by RT-PCR. LPS increased the gene expressions of cytokines in BV-2, but these increases were significantly suppressed by EPO. Next, we assessed the capacity of the phagocytosis of BV-2 using fluorescent polystyrene beads. LPS increased the phagocytosis in BV-2 compared with untreated BV-2, but this increase was significantly suppressed by EPO. *In vivo*, we investigated the effect of EPO on the brains of mice stimulated by LPS. LPS increased the gene expressions of cytokines in mouse brains. Most of the increases of cytokines were significantly suppressed by EPO. We also assessed the morphology of microglia in the brains of mice stimulated by LPS and EPO. LPS induced microglia to be activated types, but EPO alleviated the active morphological change. These data indicated that LPS made microglia activated and cytotoxic, but EPO-EPOR signal might attenuate LPS-induced microglial cytotoxic activation.

Disclosures: **T. Tamura:** None. **M. Aoyama:** None. **H. Kakita:** None. **S. Ukai:** None. **K. Sobue:** None. **K. Asai:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.08/B112

Topic: B.11. Glial Mechanisms

Support: FCT, Portugal (Project PTDC/BIM-MEC/0913/2012

Strategic Projects PEst-C/SAU/UI3282/2013

UID/NEU/04539/2013), COMPETE-FEDER

AIBILI

Title: Control of microglia reactivity elicited by elevated hydrostatic pressure through A2A receptor blockade

Authors: *A. F. AMBROSIO[1,2,3], I. AIRES[2,3], C. NEVES[2,3], R. BOIA[2,3], M. H. MADEIRA[2,3], A. R. SANTIAGO[2,3,4];
[1]AIBILI NIF 502288957, Coimbra, Portugal; [2]Inst. for Biomed. Imaging and Life Sci. (IBILI), Fac. of Medicine, Univ. of Coimbra, Coimbra, Portugal; [3]CNC.IBILI, Univ. of Coimbra, Coimbra, Portugal; [4]Assn. for Innovation and Biomed. Res. on Light and Image (AIBILI), Coimbra, Portugal

Abstract: Glaucoma is a retinal degenerative disease characterized by degeneration of retinal ganglion cells (RGCs) and damage of the optic nerve, and elevated intraocular pressure (IOP) is a major risk factor. Retinal neuroinflammation has been described in human glaucoma and in experimental models of the disease. Several reports have shown that microglia become reactive in glaucomatous eyes, which may contribute to RGC loss. Evidence shows that the blockade of adenosine A2A receptor (A2AR) affords protection against several noxious conditions, presumably through the control of microglia-mediated neuroinflammation. In this study, we aimed to assess whether blocking A2AR is able to control microglia reactivity elicited by elevated hydrostatic pressure (EHP), which mimics elevated IOP. Retinal primary neural cell cultures and microglial cell cultures (BV-2 cell line) were pre-treated with 50 nM SCH 58261, a selective A2AR antagonist, and exposed to EHP (70 mmHg above normal atmospheric pressure). Control cells were incubated in a standard cell incubator. Microglial cell morphology, phagocytosis, proliferation, migration, and the release of inflammatory cytokines were assessed. Exposure of retinal neural cell cultures to EHP for 24h increased microglia reactivity and the release of IL-1β and TNF, assessed by alteration in microglia morphology and ELISA, respectively. Furthermore, EHP increased microglia proliferation, as determined by EdU incorporation. The blockade of A2AR decreased cell proliferation and cytokine release elicited by EHP in primary retinal cultures. In BV-2 cells, exposure to EHP increased mRNA and protein levels of A2AR, as assessed by qPCR and western blotting, respectively. In addition, EHP increased microglia migration, determined by the scratch wound assay and modified Boyden chamber assay, as well as phagocytosis and the release of IL-1β and TNF. Pretreatment of BV2 microglia with SCH 58261 decreased migration, phagocytosis efficiency and cytokine release. Our results demonstrate that the blockade of A2AR is able to prevent microglia-mediated neuroinflammatory processes, controlling microglia reactivity, thus suggesting that A2AR antagonists could be envisaged as a therapeutic strategy for the management of retinal neuroinflammation in glaucoma.

Disclosures: **A.F. Ambrosio:** None. **I. Aires:** None. **C. Neves:** None. **R. Boia:** None. **M.H. Madeira:** None. **A.R. Santiago:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.09/C1

Topic: B.11. Glial Mechanisms

Support: ncRNA Pain FP7

Title: MicroRNA including exosome shuttling as a mechanism of neuron-microglia communication

Authors: *E. A. OLD, M. MALCANGIO;
Wolfson CARD, King's Col. London, London, United Kingdom

Abstract: The treatment of neuropathic pain remains problematic; patients' responses to current pharmacological interventions are often poor and associated with a number of undesirable side-effects, thus the identification of new targets for treating this debilitating condition is needed. A wealth of evidence has been obtained in recent years demonstrating the importance of neuron-glia communication in pain signaling in both its physiological and pathological form, and the manipulation of this process is proving a viable means to halt the development of chronic pain. Here we explore the contribution to neuron-glia signaling of microRNAs contained within exosomes. Exosomes are small vesicles (30-150nm) containing RNA or protein cargos that are secreted by all cells types, as such they occur endogenously in bodily fluids including blood and CSF. Indeed, whilst initially thought to be a cellular mechanism of waste disposal, exosomes are now considered to be highly specified enablers of intra- and intercellular communication, and recent evidence has indicated that exosomes can be speificically targeted to recipient cells. A number of *in vitro* and *ex vivo* approaches have been adopted to obtain the results presented here. The release of microRNAs containing exosomes has been induced from primary cultured dorsal root ganglia (DRG) neurons, and dorsal horn neurons in *ex vivo* slice mounts. These exosomes, isolated from the cell media or slice superfusates have been subjected to analysis by western blot and RT-QPCR to determine their contents. In addition to their protein or RNA rich core, exosomes are enriched in several raft-associated lipids, such as ceramide, and specific surface proteins which have these small vesicles to be immunofluroescently labeled and tracked as they are applied to and engulfed by non-neuronal cells *in vitro*. Here we report the uptake of neuron-derived exosomes by non-neuronal cells, and demonstrate that these exosomes express microRNAs likely to significantly regulate pain-associated genes following nerve injury, including the pro-inflammatory microRNAs let-7b and the pro-resolution associated microRNAs mir21. Additionally we demonstrate that the application of a noxious stimulus to neurons is able to alter the expression of microRNAs found with exosomes.

Disclosures: E.A. Old: A. Employment/Salary (full or part-time):; ncRNA Pain FP7 grant. **M. Malcangio:** A. Employment/Salary (full or part-time):; ncRNA Pain FP7 grant.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.10/C2

Topic: B.11. Glial Mechanisms

Support: NSERC Discovery Grant and Accelerator Award

Title: Acute effects of glutamate on brain and spinal microglia

Authors: *S. BASKAR JESUDASAN[1], M. CHURCHWARD[2], K. G. TODD[1], I. R. WINSHIP[1];
[1]NMHI, Neurochemical Res. Unit, Dept. of Psychiatry, [2]Neurochemical Res. Unit, Dept. of Psychiatry, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Microglia are a unique population of central nervous system (CNS) cells that, though haematopoietic in origin, play a key role in homeostasis of the CNS. Recent studies have shown microglia are involved in regulating neural progenitor populations, pruning of synapses during development, monitoring the heath of the adult CNS, and initiating responses to any perturbation in the CNS. Understanding the role of environment in moderating microglial activity will further enhance our knowledge of their role in outcome after an insult to the CNS. It has been shown that ischaemic stroke and spinal cord injury cause neuronal and glial morbidity, leading to release of neurotransmitters such as glutamate, ATP and increase in cellular debris in the extra cellular milieu. Glutamate released in the extra cellular milieu induces excitotoxic injury to neurons and chemotaxis of microglia during the early stages of ischaemia in the CNS. To what extent glutamate induces release of pro-inflammatory molecules and phagocytosis is unknown. Here, we tested the acute effects of varying concentrations of glutamate, mimicking physiological and ischaemic glutamate levels, on release of cytokines including interleukin (IL)-1β, IL-6 and tumour necrosis factor alpha (TNF-α) and phagocytic activity of microglia derived from the brain or spinal cords of neonatal rats. We found that glutamate at physiological concentrations as well as at concentrations mimicking ischaemia did not induce a significant increase in the level of TNF-α, IL-1β and IL6. However, an overall reduction in TNF-α release by spinal cord microglia was observed relative to brain microglia. Phagocytic activity of brain and spinal microglia was not significantly affected by glutamate at physiological or at concentrations mimicking ischaemia. These data suggest that glutamate is not an inducer of pro-inflammatory factors such as TNF- α, IL-1β and IL-6, and that the major role of glutamate is to recruit microglia to the site of excitotoxic injury. The inflammation observed at sites of excitotoxic injury could be due to interaction of microglia with the myriad of molecules that are released at such sites and interaction with other glial as well as infiltrating cells

Disclosures: S. Baskar Jesudasan: None. **M. Churchward:** None. **K.G. Todd:** None. **I.R. Winship:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.11/C3

Topic: B.11. Glial Mechanisms

Support: NIDA / NIH IRP

Title: Microglia establish region specific phenotypes in the basal ganglia and exhibit variable responses to normal aging

Authors: *L. M. DE BIASE[1], Z. H. FUSFELD[1], K. E. SCHUEBEL[2], K.-W. JAIR[2], H. ZHANG[1], Q.-R. LIU[1], S. P. RIBEIRO[1], R. CIMBRO[3], I. A. HAWES[1], H. SHEN[1], Z.-X. XI[1], D. GOLDMAN[2], A. BONCI[1];
[1]Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD; [3]Rheumatology, Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Microglia promote brain homeostasis by removing debris from the extracellular space and responding to diverse CNS insults. Microglia can also influence synaptic transmission through release of inflammatory and trophic signaling factors and activity-dependent phagocytosis of synapses. Although regional differences in microglial density and morphology have been reported, these cells are generally assumed to be functionally equivalent throughout the CNS. We used transgenic mice that express EGFP within microglia to quantify anatomical, electrophysiological, and molecular properties of these glia within the basal ganglia (BG), a collection of brain nuclei that regulate goal-directed behaviors and are pathologically altered during addiction and neurodegenerative disease. Immunohistochemical analysis revealed that microglial density in the nucleus accumbens (NAc) was comparable to that reported for cortex, while density in the substantia nigra pars reticulata (SNr) was markedly elevated and that in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) was significantly depressed. SNr and NAc microglia also displayed highly complex morphologies compared to VTA and SNc microglia. These anatomical distinctions were accompanied by differences in intracellular lysosome content and distribution, suggesting that microglial phagocytotic activity or metabolic state may vary across BG nuclei. Electrophysiological recordings of microglia in acute brain slices revealed that SNr microglia have more negative resting membrane potential than VTA or SNc microglia. In addition, 65% of microglia within the SNr exhibited delayed rectifier potassium currents, whereas only 9% of VTA and SNc microglia displayed such currents, which have been linked to expression of P2Y12 receptors. Whole transcriptome RNA sequencing of microglia isolated from distinct BG nuclei revealed broad similarities in gene expression between BG microglia and cortical microglia. In addition, microglia from each BG nucleus also exhibited unique gene expression signatures, challenging the idea that these cells are functionally equivalent. During the course of normal aging, region-specific differences in microglial branching complexity are largely maintained. However, abnormal soma shape and bulbous swellings along cell processes were more prominent in midbrain as compared to forebrain BG nuclei. Together these findings indicate that microglia establish region-specific phenotypes within the BG and raise important questions about the impact of this heterogeneity on neuronal function and susceptibility to neurodegeneration and other pathologies.

Disclosures: **L.M. De Biase:** None. **Z.H. Fusfeld:** None. **K.E. Schuebel:** None. **K. Jair:** None. **H. Zhang:** None. **Q. Liu:** None. **S.P. Ribeiro:** None. **R. Cimbro:** None. **I.A. Hawes:** None. **H. Shen:** None. **Z. Xi:** None. **D. Goldman:** None. **A. Bonci:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.12/C4

Topic: B.11. Glial Mechanisms

Support: CIHR (ESR)

CIHR Vanier CGS (NAIF)

Title: Early inflammation dysregulates neural circuit formation *in vivo* via microglial activation and IL-1β

Authors: *N. FAROOQI, J. P. ANTEL, E. S. RUTHAZER;
McGill Univ., Montreal, QC, Canada

Abstract: Studies of maternal infection and genome-wide association studies strongly implicate the immune system in the aetiology of neuropsychiatric disease including autism and schizophrenia. The mechanistic links between immune activation and neural circuit formation remain largely obscure. We investigate these links by acutely exposing larval zebrafish to bacterial lipopolysaccharide (LPS) and observing neural development before and after inflammation. Bath-exposure to LPS induces acute upregulation of inflammatory cytokines (TNFα, IL-1β, IL-6) and induces microglia to adopt an amoeboid morphology consistent with activation. *In vivo* two-photon microscopy of retinal ganglion cells (RGCs) after two-hour LPS exposure demonstrates acutely increased rates of axonal branch addition and retraction. Daily imaging of axons after LPS exposure, however, reveals a stunted developmental trajectory with smaller, simpler arbors and fewer presynaptic puncta. Acute structural hyperplasticity followed by reduced arborisation may suggest a failure to stabilise nascent synapses. To investigate the cellular and molecular mechanisms of these effects, we performed morpholino knockdown of the SpI1/Pu.1 transcription factor to deplete the myeloid lineage. Morphant animals lack microglia and macrophages. RGCs in Spi1/Pu.1 morphants exposed to LPS do not demonstrate either the acute or lasting perturbations of arborisation seen in wildtype LPS-treated animals, suggesting that microglial activation is necessary for these effects. Morpholino knockdown of IL-1β phenocopies the effect of myeloid depletion and administration of recombinant IL-1β mimics the effect of LPS, demonstrating that IL-1β is necessary and sufficient to mediate the developmental effects of inflammation. By indicating mechanistic links between early inflammation and neurodevelopmental processes, this work may suggest therapeutic interventions to mitigate the effects of inflammatory insult on a developing brain. Funding: CIHR Vanier CGS (NAIF); CIHR (ESR).

Disclosures: N. Farooqi: None. **J.P. Antel:** None. **E.S. Ruthazer:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.13/C5

Topic: B.11. Glial Mechanisms

Support: Canadian Institute for Health Research

Alberta Health Services

Davey Endowment for Brain Research

Alberta Innovates - Health Solutions

Title: Metabolic Stress and Survival of Microglia

Authors: *M. A. CHURCHWARD, K. G. TODD;
Psychiatry, Univ. of Alberta, Edmonton, AB, Canada

Abstract: As the primary immune cells of the central nervous system (CNS), microglia have been increasingly recognized for their contributions to development and maintenance of the CNS including activity-dependent synaptic refinement, maintenance of neural progenitor populations, and homeostatic control. In conditions of injury, infection, or duress however, microglia are responsible for surveillance, initiation of inflammatory cascades, and clearance of debris from sites of injury. As a consequence of this role microglia are capable of surviving and proliferating in conditions that are injurious to other cell types. The core of an ischemic injury is defined by the absence of blood flow resulting in oxygen and glucose deprivation, though microglia migrate to and proliferate in the ischemic core. We sought to investigate the factors contributing to the survival of primary cultured microglia in adverse conditions. Glucose deprivation (GD) for extended periods (up to 48 h) was insufficient to increase release of inflammatory factors from microglia *in vitro*, however phagocytosis was markedly increased after 24 h GD relative to normal glucose. Glucose deprivation did not adversely affect the viability of microglia: in fact increased cell counts were observed following 24 h GD, suggesting increased survival and/or proliferation in the absence of glucose. Pharmacological manipulation of lipid metabolism yielded effects that were dependent on the presence of glucose: inhibition of triglyceride biosynthesis increased both microglial viability and phagocytosis in the presence, but not absence of glucose. These data suggest microglia may survive hostile conditions in injury by metabolism of alternative energy stores including lipids in order to sustain essential protective and reparative functions.

Disclosures: M.A. Churchward: None. **K.G. Todd:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.14/C6

Topic: C.09. Demyelinating Disorders

Support: CIHR

AIHS

Title: LPS enhanced M2-polarized macrophages/microglia promote robust remyelination in lysolecithin demyelination injury

Authors: ***M. K. MISHRA**, K. RAWJI, M. B. KEUOGH, Y. FAN, V. W. YONG;
Clin. Neurosci., Univ. of Calgary, Calgary, AB, Canada

Abstract: Macrophages and CNS-resident microglia are broadly classified into pro-inflammatory M1 and anti-inflammatory M2 subtypes, and they may switch from one state to the other depending on the microenvironment. Using well-described culture conditions, specifically interferon-γ/lipopolysaccharide (LPS) and IL-4/IL-13 respectively, we have generated M1 and M2 cells in order to address their effects on neurons and oligodendrocyte precursor cells (OPCs) in culture, on altering the functions of the chondroitin sulfate proteoglycans (CSPGs) implicated in inhibiting remyelination, and on mediating recovery from demyelination in mice. Unexpectedly, we discovered that the LPS stimulation of M2 cells during their polarization further enhanced M2 characteristics and their beneficial properties, in part through elevation of IL-4 receptor and IL-10 expression. The LPS-enhanced M2 cells did not kill neurons and OPCs in culture, unlike the M1 cells generated by interferon-γ and LPS. To investigate the impact of polarized macrophages/ microglia *in vivo*, we inflicted demyelination of the dorsal column of the mouse spinal cord using lysolecithin; 3 days after, saline, M1 and M2-polarizing conditions, or M2+LPS were locally applied at the site of injury. Treatment that generated M1 exacerbated injury, M2 had negligible effect on the evolving insult, while the LPS-enhanced M2 treatment diminished the accumulation of inhibitory CSPGs, increased the number of oligodendrocyte precursors, and reduced lesion volume. LPS enhanced M2 treatment promotes remodelling at the lesion microenvironment that results in robust remyelination at day 21 after lysolecithin demyelination. These data suggest that toll-like receptor-4 stimulation of M2 cells during their polarization can dramatically enhance their beneficial M2 characteristics to favor recovery from CNS insults.

Disclosures: **M.K. Mishra:** None. **K. Rawji:** None. **M.B. Keuogh:** None. **Y. Fan:** None. **V.W. Yong:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.15/C7

Topic: C.09. Demyelinating Disorders

Support: NIH IRACDA NY-CAPS

NMSS CA1044A1

NMSS PP1815

NIH R01NS42168

Title: Pharmacological depletion of microglia attenuates experimental autoimmune encephalomyelitis

Authors: *J. NISSEN, S. E. TSIRKA;
Pharmacol., Stony Brook Univ., Stony Brook, NY

Abstract: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) that is characterized by the progressive demyelination and degeneration of neurons. As MS progresses, T cells that are normally excluded from the CNS cross the compromised blood-brain barrier due to recruitment by the resident CNS immune cells, microglia. These microglia then become activated and release a variety of inflammatory cytokines that greatly contribute to disease progression. Thus, therapeutic approaches in MS focus on diminishing inflammation and promoting expansion of anti-inflammatory immune cell populations. Here, we show that drug-based ablation of microglia significantly improves symptoms in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Further, we show that this treatment addresses the pathological hallmarks of MS as well, for ablation of microglia reduces demyelination and immune activation. Taken together, these findings indicate that manipulation of microglial numbers is a promising treatment for MS.

Disclosures: J. Nissen: None. **S.E. Tsirka:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.16/C8

Topic: C.09. Demyelinating Disorders

Title: *In vivo* activating microglia imaging in multiple sclerosis using PET with DPA-713

Authors: *S. KONO[1], T. TERADA[2], Y. OUCHI[2], H. MIYAJIMA[1];
[2]Dept. of Biofunctional Imaging, Med. Photonics Res. Ctr., [1]Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan

Abstract: *In vivo* activating microglia imaging in multiple sclerosis using PET with DPA-713 Abstract *Introduction:* Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Activated microglia and macrophages play a key role in the immunopathogenesis of MS. Microglia activation is associated with elevated expression of the 18 kDa mitochondrial translocator protein (TSPO). This study evaluated imaging of activated microglia in MS patients using the PET tracers [^{11}C]-DPA-713, TSPO-specific radioligands. *Methods:* Six relapsing and remitting type of MS patients (mean age 38.8 ± 10.8) in the early stage of disease (EDSS 1 or 2) and six age-matched normal controls (37.8 ± 22.3) underwent [^{11}C]-DPA713-PET scan. The MS patients were treated with disease modifying therapy including interferon β1b (IFNβ1b) and fingolimod. All patients were followed-up and the second PET scan was performed one year later. The binding potential (BP_{ND}) was estimated using simplified reference tissue model. Statistical Parametric Mapping (SPM) was used to compare regional BP_{ND} levels between the MS and control groups. *Results:* All the MS patients displayed a significant increase of DPA BP_{ND} in the left cerebellum, the bilateral putamen, the bilateral cerebral area, and brainstem at the first PET scan in the ROI analysis. Accumulation of DPA was observed in cortical area as well as white matter which are independent from demyelinating plaques. Furthermore, increases in [11C]-DPA713 BP were found more broadly in the whole brain at the second PET scan in the SPM analysis. There were no difference in change of DPA BP_{ND} in the MS patients with treatment with fingolimod and IFN1b *Conclusion:* The DPA-PET study indicates that activated microglia is implicated in the normally appearing white matter and cortical area even in the early stage of MS. The effect of disease modifying therapy can be monitored *in vivo* by measuring the degree of microglial activation on DPA-PET.

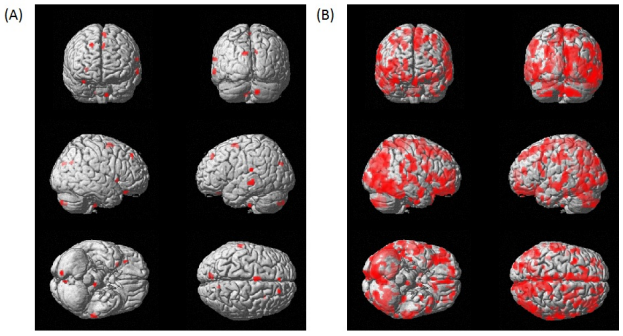


Figure legend
The regions of statistically significant increase in [11C]DPA binding potential in Multiple Sclerosis patients group (A) at first scan compared to age-matched controls group, and (B) at second scan after one year later (p<0.001, uncorrected, extend threshold 10).

Disclosures: **S. Kono:** None. **T. Terada:** None. **Y. Ouchi:** None. **H. Miyajima:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.17/C9

Topic: B.11. Glial Mechanisms

Support: NIH Grant D024044

NIH Grant DE107782

NIH Grant DA023132

NHMRC Grant 1054091

Title: Selective manipulation of spinal microglia by chemogenetics: implications for allodynia and inflammatory signaling

Authors: *P. M. GRACE[1], X. WANG[1], D. J. URBAN[2], M. V. BARATTA[1], E. L. GALER[1], K. A. STRAND[1], Y. ZHANG[1], H. YIN[1], B. L. ROTH[2], S. F. MAIER[1], L. R. WATKINS[1]; [1]Psychology and Neurosci., Univ. of Colorado, Boulder, Boulder, CO; [2]Univ. of North Carolina, Chapel Hill, NC

Abstract: The absence of selective pharmacological tools is a major barrier to the *in vivo* study of microglia. To address this issue, we developed a Gq- and Gi-coupled Designer Receptor Exclusively Activated by a Designer Drug (DREADD) to enable selective stimulation or inhibition of microglia, respectively. DREADDs under a CD68 (microglia/macrophage) promoter were intrathecally transfected via an AAV9 vector. Naïve rats intrathecally transfected with Gq (stimulatory) DREADDs exhibited significant allodynia following intrathecal administration of the DREADD-selective ligand clozapine-N-oxide (CNO), which was abolished by intrathecal interleukin-1 receptor antagonist. Chronic constriction injury-induced allodynia was attenuated by intrathecal CNO in rats intrathecally transfected with Gi (inhibitory) DREADDs. To explore mechanisms, BV2 cells were stably transfected with Gq or Gi DREADDs *in vitro*. CNO treatment induced pro-inflammatory mediator production per se from cells expressing Gq-DREADDs, and inhibited lipopolysaccharide-induced pro-inflammatory mediator production from cells expressing Gi-DREADDs. These studies are the first to manipulate microglia using DREADDs, which allow the role of glia in pain to be conclusively demonstrated, unconfounded by neuronal off-target effects that exist for all other glial inhibitors. Hence, these studies are the first to conclusively demonstrate that *in vivo* stimulation of resident spinal microglia in intact spinal cord is a) sufficient for allodynia, and b) necessary for allodynia induced by peripheral nerve injury. DREADDs are a unique tool to selectively explore the physiological and pathological role of microglia *in vivo*.

Disclosures: **P.M. Grace:** None. **X. Wang:** None. **D.J. Urban:** None. **M.V. Baratta:** None. **E.L. Galer:** None. **K.A. Strand:** None. **Y. Zhang:** None. **H. Yin:** None. **B.L. Roth:** None. **S.F. Maier:** None. **L.R. Watkins:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.18/C10

Topic: B.11. Glial Mechanisms

Support: NIH R21 OD016562

NIH R01 MH093595

Title: Intracellular calcium dynamics in cortical microglia responding to focal laser injury

Authors: ***P. TVRDIK**, A. POZNER, B. XU, S. PALUMBOS, J. GEE, M. R. CAPECCHI; Human Genet., Univ. of Utah, Salt Lake City, UT

Abstract: Microglia are highly reactive to tissue injury. In response to focal damage, microglia extend their processes toward the compromised tissue. The cell motility aspects of this behavior have been characterized, but the intracellular events, particularly the Ca2+ responses, have not been systematically studied in microglia due to technical difficulty. Here we used live two-photon imaging in the PC::G5-tdT reporter mouse expressing the genetically encoded Ca2+ indicator GCaMP5G and fluorescent marker tdTomato in cortical microglia. We found that spontaneous Ca2+ transients in microglial somas and processes were generally low (only 4% of all microglia showing transients within 20 min), but baseline activity was markedly increased when the animals were subcutaneously injected with LPS 12 h before imaging. When challenged with focal laser injury, an additional surge in Ca2+ activity (up to 67%) was observed in the somas and protruding processes, particularly in close proximity to the damaged tissue. Notably, coherent and simultaneous Ca2+ rises in multiple microglial cells were occasionally detected in LPS- and bicuculline-treated animals. We have demonstrated that Ca2+ transients were predominantly mediated via purinergic receptors. We also compared the speed of process extension in BAPTA-AM- and the purinergic receptor antagonist PPADS-treated animals and showed that BAPTA-AM significantly slowed the process protrusion, but PPADS did not. This study illustrates the suitability of the PC::G5-tdT mouse reporter for investigations of microglial physiology.

Disclosures: **P. Tvrdik:** None. **A. Pozner:** None. **B. Xu:** None. **S. Palumbos:** None. **J. Gee:** None. **M.R. Capecchi:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.19/C11

Topic: C.09. Demyelinating Disorders

Support: Don and Fran Herdrich

Moon and Marilyn Park

Title: Macrophages and microglia that phagocytose myelin debris acquire a unique activation status and produce factors that stress axons

Authors: *M. M. STANDIFORD, C. L. HOWE;
Mayo Grad. Sch., Rochester, MN

Abstract: There are several treatments for multiple sclerosis (MS) that reduce relapse rate and lesion accumulation on MRI, however, at present none of these therapies are of proven benefit in reducing the progression of long-term clinical disability. Although MS is characterized as a demyelinating disorder, neurologic disability associated with the disease is correlated with markers of axonal injury rather than markers of demyelination. This suggests that damage to axons is the principal cause of irreversible disability in MS and that the mechanisms underlying demyelination and axon injury may be fundamentally different from one another. Nonetheless, we know that demyelination is a necessary, albeit insufficient, condition for axon injury in MS. We therefore tested the hypothesis that macrophages and microglia exposed to myelin debris acquire an activation phenotype that creates an environment within demyelinated lesions which, in turn, drives immunological recognition of denuded axons. On axons cultured in microfluidic chambers, we have observed that interferon gamma induces upregulation of MHC class I molecules loaded with endogenous neoantigen peptides and that this renders the axons susceptible to perforin- and granzyme-dependent injury by antigen-specific CD8+ T cells. We have also observed that myelin debris triggers a unique activation pattern in macrophages and microglia that is consistent with the production of cytokines that drive MHC class I expression on axons. This activation phenotype has a blend of traditional M1 and M2 characteristics and is unique to myelin stimulation. We conclude that demyelination results in immune-mediated axon injury via an indirect mechanism that involves macrophage and microglial activation by myelin debris.

Disclosures: M.M. Standiford: None. **C.L. Howe:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.20/C12

Topic: C.09. Demyelinating Disorders

Support: Emerald Foundation 2013-2015 Award.

Title: CSF from progressive MS patients stimulates microglial activation pathways *in vitro* and *in vivo*

Authors: *M. CRISTOFANILLI, K. MCDERMOTT, B. PAGANO, D. GRATCH, S. A. SADIQ;
Tisch MS Res. Ctr. of New York, New York, NY

Abstract: Microglia are resident immune cells in the CNS that act as macrophages when activated by injury or disease. This activation is a characteristic of inflammatory diseases like multiple sclerosis (MS) in which two main phenotypes occur: classically activated pro-inflammatory M1 and alternatively activated anti-inflammatory M2. However, the exact role of M1/M2 cells in MS is not fully understood. In this study, we investigated the effect of MS cerebrospinal fluid (CSF) on microglial activation using our recently published mouse model of progressive MS. In this model, mice develop inflammatory demyelinating brain lesions after serial intracerebroventricular injections of acellular CSF obtained from untreated progressive MS patients. Here we injected mice with CSF from 7 primary progressive (PP), 7 secondary progressive (SP), and 5 relapsing remitting (RR) MS patients, as well as from 4 non-MS inflammatory controls (IC) and 2 healthy individuals (HC). Microglia/macrophages were extracted from mice brains (n=3 per patient) with a Percoll gradient for FACS analysis. Compared to naïve mice or mice injected with artificial CSF, animals in all other groups displayed an upregulation of CD11b+/CD45high macrophages. In addition, the number of these cells was significantly higher in the PP and SP groups than controls. Remarkably, CSF derived from progressive MS patients who were clinically stable following therapy had greatly diminished capacity to activate microglia/macrophages compared to the paired untreated samples. To further investigate the mechanism of CSF-induced microglial activation, we stimulated BV-2 cells, a homogenous murine cell line expressing phenotypical and functional markers of macrophages, with the same CSF cohort described above. FACS and q-PCR analysis were used to asses M1/M2 polarization. The number of M1 macrophages was significantly increased in the PP group compared to the RR and control ones. A similar trend was found for the SP group without statistical significance. M2 macrophages were significantly higher in both the PP and HC groups compared to the RR and IC ones. Once again, the SP group showed a similar trend to the PP group without statistical significance. Interestingly, M1 were more abundant than M2 for each group with the exception of the HC treated samples, which had an even cell distribution. In conclusion, our preliminary evidence suggests the existence of intrinsic differences related to microglial activation between MS and non-MS patients and within MS

subtypes. Future studies will aim to pinpoint specific factors in the progressive MS milieu responsible for the reported microglia polarization toward M1/M2 phenotypes.

Disclosures: M. Cristofanilli: None. **K. McDermott:** None. **B. Pagano:** None. **D. Gratch:** None. **S.A. Sadiq:** None.

Poster

298. Microglia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 298.21/C13

Topic: B.11. Glial Mechanisms

Title: Triggering a moderate brain inflammatory response by MPL or Pam3Cys preserve normal performance in a rat model of Alzheimer's disease

Authors: *H. G. BADIE[1,2], M. SAYYAH[2], B. KHOSHKHOLGH-SIMA[2], S. CHOOPANI[2], M. SHOKRGOZAR[3];
[1]Neurosci. Res. Ctr., Neurosci. Res. Ctr., Tehran, Iran, Islamic Republic of; [2]Physiol. and Pharmacol., [3]Pasteur Inst. of Iran, Tehran, Iran, Islamic Republic of

Abstract: Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of amyloid β (Aβ) in brain parenchyma. Inflammation along with the progression of the disease, is associated with the production of cytokines by activated microglia. Although Aβ oligomers fail to fully activate microglia, Aβ plaques can trigger the exaggerated release of cytokines and neurotoxic mediators which could be detrimental to neurons. On the other hand, activated microglia can clear Aβ via increased phagocytosis and proteolytic degradation, which may be neuroprotective. Toll-like receptors (TLRs), especially TLR2 and TLR4, on the surface of microglial cells play a critical role in clearance of Aβ. Therefore, in this study the possible neuroprotective effect of pretreatment with agonists of these receptors were examined. Methods: Pretreatment with MPL (Monophosphoryl lipid A) and Pam3Cys, partial agonists for TLR4 and TLR2 respectively, were examined in cell culture and *in vivo* to indicate whether they could prevent or attenuate the symptom of AD in a rat model of AD by implanting Alzet pump filled with Aβ. Results: In the cell culture experiment, we found that whereas Aβ oligomers did not significantly stimulate BV-2 cells to produce cytokines, pretreatment with MPL or Pam3Cys led to increase release of TNF-α, IL6 and IP10. However,their levels were less than those pre-retriggered by LPS. In western blot, MPL and Pam3CYS increased the expression of FPR2 protein which is involved in clearance of Aβ. AD animals pretreated with MPL and Pam3Cys showed improved reference spatial and working memory. However, pretreatment by LPS has not improved behavioral performance in these rats. Conclusion: Further studies are needed in this context, however, our data provide an early promising result in preventing AD by triggering an early and specific moderate neuroinflammatory reaction.

Disclosures: **H.G. Badie:** A. Employment/Salary (full or part-time):; Physiology and Pharmacology Department, Pasteur Institute of Iran, Tehran, Iran. **M. Sayyah:** None. **B. Khoshkholgh-sima:** None. **S. Choopani:** None. **M. Shokrgozar:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.01/C14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC 2012-81260650

Title: The effect of polysaccharide of cistanche deserticola on synaptic plasticity in mice

Authors: ***G. LI**, M. GUO, H. ZHANG, Y. WU, R. YIN;
Inner Mongolian Med. Univ., Hohhot, China

Abstract: Cistanche deserticola MA is a “Yang-invigorating” tonic herb in Chinese medicine. We isolated a polysaccharide from C. deserticola (CDP), studied the effect of the polysaccharide on memory acquisition impairment. Aim: To study the effect of polysaccharide of Cistanche deserticola (CDPS) on synaptic plasticity and in studying and learning. Methods: Memory acquisition impairment model in mouse was established with scopolamine,the improvement in the memory acquisition impairment of mouse after was observed by step-down test and morris water maze assays.The ultra-thin specimens of hippocampus were observed under transmission electron microscope. Western blotting analysis were used to examined the expression of PSD-95,GAP-43 and SYP, respectively. Results: polysaccharide of Cistanche deserticola(50mg/kg,100mg/kg)could significantly reduce the number of errors and extend the incubation period in the step-down test.CDP (50mg/kg) could shorten the incubation period of female mouse in the water maze test. polysaccharide of Cistanche deserticola 100mg/kg could shorten the incubation period of male mouse in the water maze test. The ultra-thin specimens of hippocampus: 1)In the sham-operated group,the neuron and neuropil of hippocampus showed ultrastractual detail of normal range. 2)In the model group , the number of synapse was reduced,perforated or dysmorphic synapses were observed,and synapse vesicles were destroyed to be homogenized. We can see from the results of western blot that the expression of PSD-95,GAP-43 and SYP in hippocampus of mouse were increased significantly.Conclusion:Our findings suggest that polysaccharide of Cistanche deserticola markedly improved the behavior-learning and memory in mouse,which may be related to improvememt of the plasticity of synaptic morphology.

Disclosures: **G. Li:** None. **M. Guo:** None. **H. Zhang:** None. **Y. Wu:** None. **R. Yin:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.02/C15

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neurochemical characterization of xanomeline and a selective muscarinic M4 receptor positive allosteric modulator in the rat and non-human primate

Authors: *M. KANDEBO[1], J. MARCUS[2], M. STRANIERI MICHENER[3], B. E. SMITH[3], S. M. SMITH[4], L. YAO[4], J. A. MORROW[1];
[1]Late Discovery Psychiatry & Psychosis, [2]Late Discovery AD Cognition & Sleep, [3]Clin. Services, [4]Early Discovery Biol., Merck & Co., Inc., West Point, PA

Abstract: Xanomeline is a M1/M4 preferring muscarinic receptor agonist that has shown to alleviate behavioral (psychosis) and cognitive disturbances in Alzheimer's disease and schizophrenia patients. Xanomeline, however, has limited clinical use because of its adverse parasympathetomimetic effects associated with its muscarinic subtype selectivity profile. Preclinical data has shown that the antipsychotic-like profile of xanomeline is mediated predominantly by activation of striatal muscarinic M4 receptors in D1 receptor expressing direct pathway medium spiny neurons. Selective M4 muscarinic agonists and positive allosteric modulators demonstrate equivalent therapeutic potential to xanomeline in preclinical models of psychosis however with reduced propensity for adverse effects. To further elucidate the M1 versus M4 muscarinic receptor mediated effects of xanomeline, catecholamine profiles in the cerebrospinal fluid of non-human primate (NHP) were determined following single dose administration of xanomeline and Compound A, a selective M4 positive allosteric modulator. 0.2mg/kg dose of xanomeline and a 1mg/kg dose of Compound A resulted in significant accumulation of DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in NHP CSF. Neither xanomeline nor Compound A had a significant effect on DA concentrations. Furthermore, xanomeline, but not Compound A administration, significantly increased norepinephrine concentrations in NHP CSF suggesting this effect may be mediated through M1 muscarinic receptors. Conversely, Compound A, but not xanomeline administration, significantly decreased histamine (HA) and tele-methylhistamine (t-MeHA) in the NHP CSF. Further *in vivo* neurochemical characterization in rodents produced similar effect of xanomeline and Compound A on accumulation of DA metabolites in the striatum of Sprague Dawley rat with no effect on DA concentrations. Additionally Compound A attenuated the effects of Thioperamide, a H3 antagonist, on t-MeHA accumulation in the striatum of Sprague Dawley rat, providing additional evidence for the role of M4 muscarinic receptors in the modulation of histaminergic neurotransmission. These results demonstrate that xanomeline and selective M4 PAMs possess similar neurochemical profiles in terms of their effects on DA and DA metabolites but differentiate in terms of their effects on NE and HA/t-MeHA. Together, these

findings provide an insight into M4 muscarinic receptor mediated DA signaling and are the first to provide evidence of a role for M4 muscarinic receptors in modulation of histaminergic neurotransmission.

Disclosures: **M. Kandebo:** None. **J. Marcus:** None. **M. Stranieri Michener:** None. **B.E. Smith:** None. **S.M. Smith:** None. **L. Yao:** None. **J.A. Morrow:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.03/C16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Doctoral Fellowship

NSERC

Title: Role of Somatostatin in RA induced neurogenesis of SH-SY5Y cells and βA induced toxicity

Authors: S. PAIK, R. K. SOMVANSHI, *U. KUMAR;
Pharmaceut. Sci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Introduction: Somatostatin (SST, SRIF) is a growth hormone inhibitory peptide first isolated from hypothalamus. SST is widely expressed in different body parts and is also produced in neuroendocrine cells and regulates variety of physiological functions including hormonal secretion and cell proliferation. Previous studies have reported that the SST is one of the most consistently down-regulated genes across all Alzheimer's Disease (AD) patients, both in post-mortem brain and in cerebrospinal fluid (CSF). Consistent with these findings, our lab has also observed significant loss (>70%) of SST-immunoreactive neurons in the frontal cortex of AD brains. In the present study, we aim to elucidate the exact role of SST in neurogenesis of human-derived neuroblastoma cell line as well as to study its protective role against β-Amyloid (βA) induced toxicity. Methods: SH-SY5Y cells were differentiated with 10μM of retinoic acid (RA) with or without SST (concentration and time dependent manner) for 5-7 days. Post treatment cells were processed for the expression of SST receptor (SSTR) subtypes and neuronal markers including DCX (doublecortin), GFAP (Glial fibrillary acidic protein) and MAP2 (Microtubule-associated protein 2) at the level of mRNA and protein using qRT-PCR, Western blot and immunofluorescence immunocytochemistry. Also, βA induced toxicity in non-differentiated and/or differentiated cells were determined using MTT assay in absence or presence of SST. Results: Morphological analysis revealed significant elongation of neurites in RA-induced neuro-differentiation which was further enhanced in presence of SST in concentration dependent manner when compared to RA treatment alone. Furthermore, qRT-PCR

and western blot analysis support promotion of neurogenesis in presence of SST as indicated by increased expression of DCX, GFAP and MAP2 as well as loss of Nestin (marker for stemness). In addition, MTT assay and Hoechst dye staining clearly indicate protective role of SST in βA induced toxicity and inflammation in concentration and time dependent manner. Conclusion: Taken together, the results presented here indicate that SST enhanced RA mediated neurite elongation and markers of neurogenesis as well as exert a neuroprotective role against βA induced neurotoxicity.

Disclosures: S. Paik: None. **R.K. Somvanshi:** None. **U. Kumar:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.04/C17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant-in-Aid for scientific Research (C) from the Japanese Society for the Promotion of Science

Kumamoto Health Science University special fellowship (grant number 2014-C-01)

HIGO Program Research Funding Project FY2014 By Kumamoto University Program for Leading Graduate Schools

Title: Centrally acting non-narcotic antitussives improve Aβ25-35 induced cognitive deficits in mice

Authors: *R. KAWAHARA[1,2], F. SOEDA[1], S. MISUMI[1], K. TAKAHAMA[3,4];
[1]Dept. Env. Mol. Health. Sci. Grad. Sch. Pharm. Sci., Kumamoto Univ., Kumamoto, Japan; [2]Program for Leading Grad. Sch. "HIGO Program", Kumamoto Univ., Kumamoto, Japan; [3]Res. Inst. for Drug Discovery Sch. of Pharm. Kumamoto Univ., Kumamoto, Japan; [4]Kumamoto Hlth. Sci., Univ., Kumamoto, Japan

Abstract: In spite of high morbidity rate among the elderly, there is no effective treatment capable of slowing the progression of Alzheimer's disease (AD). A few drugs for AD, such as acetylcholine esterase inhibitor and N-methyl-D-aspartate receptor antagonist, has been developed and used in clinic, although the efficacy seems not to be enough. We previously reported that centrally acting antitussives inhibit G-protein-coupled inwardly rectifying potassium (GIRK) channel currents in brain neurons. Interestingly, these drugs at antitussive effective doses ameliorated symptoms of the animal models of various intractable brain diseases. We also demonstrated that donepezil, therapeutic drug of AD, at relatively low concentrations inhibits GIRK channel current. Recently, it was reported that selective activation of dopamine

D_1/D_5 receptors protects neurons from synaptic dysfunction induced by amyloid β (Aβ) oligomers. In addition, environmental enrichment mitigates cognitive deficits in APP and APP/PS1 mice and increases the dopamine level in the nucleus accumbens. These reports suggest that activation of dopamine system in the brain may be important for effective therapy for AD. Interestingly, we revealed that antitussives increase the dopamine level in the prefrontal cortex and nucleus accumbens in rodents. According to these findings, antitussives possessing GIRK channel inhibiting action may be a potential candidate of effective therapeutic drugs for AD, having the novel mechanism of the action. In this study, therefore, we examined an effect of tipepidine and cloperastine, possessing GIRK channel inhibiting action, on $A\beta_{25-35}$ injected mice known as one of the useful models of AD. We used male ICR mice (5-week-old). Mice were intra-cerebroventricularly (i.c.v.) injected vehicle (3.2 μL) or $A\beta_{25-35}$ (3 nmol/3.2 μL), according to the method of Haley and McCormick (1957). Then, we performed the novel object recognition test (NORT) on days 5-8 after i.c.v. injection. Tipepidine and cloperastine at 40 mg/kg were subcutaneously administered 30 min before the training session of the NORT. Both drugs reversed to the same extent $A\beta_{25-35}$ induced decrease in discrimination of index in the retention session of the NORT, suggesting that tipepidine and cloperastine may have antiamnesic like effects. Pharmacological mechanisms of antiamnesic like effects of both drugs are now under investigation.

Disclosures: **R. Kawahara:** None. **F. Soeda:** None. **S. Misumi:** None. **K. Takahama:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; TAISHO PHARMACEUTICAL CO., LTD..

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.05/C18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS076815

Title: CB2 receptors are not required in ameliorating Alzheimer’s disease neuropathology by inhibition of MAGL

Authors: J. ZHANG[1], *C. CHEN[2];
[1]Neurosci. Ctr., [2]LSU Hlth. Sci. Ctr., New Orleans, LA

Abstract: We and others reported previously that inhibition of monoacylglycerol lipase (MAGL), the primary enzyme that metabolizes the endocannabinoid 2-arachidonoylglycerol (2-

AG) in the brain, produces profound anti-inflammatory and neuroprotective effects and improves synaptic and cognitive function in mouse models of Alzheimer's disease (AD). However, the molecular mechanisms underlying the beneficial effects produced by inhibition of 2-AG metabolism are still not clear. CB2 receptors are primarily expressed in astroglial cells in the brain and play an important role in neuroinflammation. To determine whether CB2 receptors are involved in ameliorating AD neuropathology produced by MAGL inactivation, we created amyloid precursor protein (APP) transgenic mice lacking the CB2 receptor (TG-CB2 KO). The results show that expression of APP, β-site amyloid precursor protein cleaving enzyme 1 (BACE1) and formation of total Aβ, Aβ42 and CTFβ/α were still elevated in TG-CB2 KO mice when compared with those in wild-type (WT)-CB2 KO mice. The elevation was reduced by JZL184, a selective and potent inhibitor for MAGL. To determine whether suppression of neuroinflammation by inhibition of 2-AG metabolism is mediated via CB2 receptors, expression of GFAP, an astrocytic marker, was detected in TG-CB2 KO mice. GFAP immunoreactivity in the brains was reduced in TG-CB2 KO mice that received JZL184. Similarly, the number of degenerated neurons in the brain was decreased in animals treated with JZL184. Importantly, spatial learning and memory assessed by the Morris water maze were significantly improved in TG-CB2 KO mice treated with JZL184 when compared with that of vehicle controls. In addition, reduced expression of glutamate receptor subunits in TG-CB2 KO mice was rescued by treatment with JZL184. Our results suggest that CB2 receptors do not play an important role in alleviating neuropathology and improving cognitive function produced by inhibition of 2-AG metabolism.

Disclosures: J. Zhang: None. **C. Chen:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.06/C19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Natural Sciences Foundation of China (No.81473200 & 81373387)

National Science and Technology Major Special Project on Major New Drug Innovation of China (2012ZX09301002-004)

2010 Program for New Century Excellent Talents in University (NCET-10-0961)

Title: L-3-n-butylphthalide improves cognitive impairment in APP/PS1-AD transgenic mouse model by enhancing neurogenesis

Authors: *Y. PENG, H. LEI, Y. ZHANG, S. XU, J. LI, L. WANG, X. WANG;
Inst. of Materia Medica, Beijing, China

Abstract: Alzheimer's disease (AD) is an age-related and irreversibly progressive neurodegenerative disorder that occurs gradually and results in memory, behavior and personality changes. L-3-n-butylphthalide (L-NBP), an extract from seeds of *Apium graveolens Linn* (Chinese celery), has been demonstrated to have neuroprotective effects on ischemic, vascular dementia and amyloid-ß (Aß)-infused animal models by inhibiting oxidative injury, neuronal apoptosis and glial activation, regulating APP processing and reducing Aß generation. In the current study, we examined the effect of L-NBP on learning and memory deficits in a APP/PS1-AD transgenic mouse model 3-month-old transgenic mice were given 15mg/kg L-NBP by oral gavage for 3 months. After behavioral testing was completed, the mice were sacrificed. The brains were removed for immunohistochemistry and Western blot. The results showed that L-NBP treatment significantly improved the spatial learning and memory deficits compared to the vehicle-treated APP/PS1 mice. L-NBP treatment significantly reduced the diffuse and compacted fibrillar Aβ plaques in the hippocampus. Furthermore, we found that L-NBP markedly enhanced soluble amyloid precursor protein secretion (αAPPs) release, but had no effect on the level of steady-state full-length APP. The effect of L-NBP on decreasing Tau phosphorylation at Thr231 was significant. L-NBP treatment significantly reduced Iba-1 immunoreactivity compared to the vehicle-treated APP/PS1 mice. Furthermore, L-NBP markedly increased $BrdU^+$ cells in hippocampal DG region and partially reversed the down-regulation of neurotrophin expressions compared to the vehicle-treated APP/PS1 mice. Moreover, L-NBP markedly enhanced pTrkA(Y490)/TrkB(Y516),pCREB,pAkt and pSTAT3 expression. In addition, we checked whether L-NBP regulated neuronal stem cells (NSCs) proliferation, migration, and differentiation *in vitro*. The results showed that L-NBP increased the total number of neurospheres and the number of large neurospheres (greater than 60μm in diameter), and enlarged the migration distance of neurospheres. L-NBP raised the number of Tuj-1 positive neurons, and non-significantly decreased GFAP positive astrocytes amount, indicating that L-NBP might induce the neuronal differentiation of NSCs toward mature neurons. Taken together, L-NBP shows promising preclinical potential as a multi-target drug for the prevention and/or treatment of Alzheimer's disease.

Disclosures: **Y. Peng:** None. **H. Lei:** None. **Y. Zhang:** None. **S. Xu:** None. **J. Li:** None. **L. Wang:** None. **X. Wang:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.07/C20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CAPES

CNPq

FAPESC

SfN Latin American Training Program

Title: Transient receptor potential ankyrin 1 (TRPA1) is important for AβO-induced toxicity: a new perspective for Alzheimer's disease?

Authors: *M. A. BICCA[1,2], E. C. S. SANTOS[1], K. L. VIOLA[2], G. LOCH-NECKEL[1], W. L. KLEIN[2], J. B. CALIXTO[1];
[1]UFSC - Univ. Federal de Santa Catarina, Florianopolis, Brazil; [2]Neurobio., Northwestern Univ., Evanston, IL

Abstract: Alzheimer's disease (AD) is a neurodegenerative and progressive disease for which there is no current cure or effective treatment. The search for new targets that could be useful models for drug development is indispensable. TRPA1 arises as a new approach, first considered by our research group to be related to AD. Endogenous molecules that activate the TRPA1 receptor (Reactive Oxygen Species (ROS), Ca2+ and products of inflammation) are also up-regulated during the initiation and progression of AD. We aimed to investigate the possible role of TRPA1 in experimental models of AD. In order do to that, we used primary neuronal cell culture from rat cortex (CT) and hippocampus (HP), Swiss mice (3 months-old) injected i.c.v. with Amyloid-β-oligomers (AβO) and also transgenic 5XFAD mouse model of AD. Cells and animals were treated with TRPA1 antagonist (HC030031) at different concentrations and schedules according to the protocol (UFSC Protocol: PP00625/2011; NU Protocol: 2014-3406). Results showed that TRPA1 is expressed in neurons and microglia but not astrocytes. AβOs induced TRPA1 redistribution and up-regulation in neurons and microglia, respectively. TRPA1 blockage in neuronal cells with HC030031 prevented AβO-induced ROS formation, mitochondrial membrane disturbance and neuronal death. Notably, when we evaluated TRPA1 expression in Swiss mice CT and HP by immunohistochemistry we observed higher expression after 24h and 7 days after AβO-treatment in the microglia and surrounding the neuronal cell body, while blot results confirmed quantifications. 5XFAD brains slices (7 m.o.) also shown up-regulated TRPA1 in CT and HP, specially surrounding amyloid plaques (AP). Of note, HC030031 pre-treatment prevented AβO-induced memory deficits in AβO injected Swiss mice while oral treatment (60 days, once a day) ameliorated memory deficits in the 5XFAD mice when compared to vehicle-treated mice. Besides, 5XFAD treated with HC030031 presented low levels of burden AP and oligomers. In summary, we are reporting for the first time the substantial role of TRPA1 in AD related experimental models. Our data suggest TRPA1 as a potential attractive target for the AD therapeutics. Authors thank CNPq, CAPES and FAPESC for the financial support.

Disclosures: **M.A. Bicca:** None. **E.C.S. Santos:** None. **K.L. Viola:** None. **G. Loch-Neckel:** None. **W.L. Klein:** None. **J.B. Calixto:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.08/C21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GACR project P303/12/0611

Title: Therapeutic effect of novel cholinesterase inhibitor 6-chlortacrine on cognitive deficit induced by 3-quinuclidinyl benzilate in rats performing the water maze task

Authors: *J. MISIK[1], M. HRABINOVA[3], J. KORABECNY[2], E. NEPOVIMOVA[2], O. SOUKUP[2], K. KUCA[4], J. KASSA[2];
[1]Fac. of Military Hlth. Sci., Czech Republic; [2]Fac. of Military Hlth. Sci., Hradec Kralove, Czech Republic; [3]Fac. of military health sciences, Hradec Kralove, Czech Republic; [4]Fac. Hosp., Hradec Kralove, Czech Republic

Abstract: The cognitive decline observed in patients suffering from Alzheimer's disease could be mitigated using cholinesterase inhibitors. In this preliminary study, pro-cognitive effect of newly-developed cholinesterase inhibitor 6-chlortacrine was investigated. 6-chlortacrine was administered to male Wistar rats in three different therapeutic doses (corresponding to 10, 20 and 40% of LD50) and the effect of the compound on 3-quinuclidinyl benzilate (QNB)-induced spatial navigation deficit was observed in water maze task. Brain cholinesterase inhibition of tested doses was evaluated in separate *in vivo* experiment using spectrophotometric method. 40 rats were divided into 5 groups of 8 animals and subjected to the WM acquisition test with a hidden platform performed as a one day session with 10 consecutive trials (swims) and an inter-trial interval of 15 min. The location of the submerged platform remained unchanged during whole sessions whereas starting positions varied randomly between trials. Each trial was videotaped and analyzed with an image tracking system (TSE VideoMot2, Bad Homburg, Germany) that provided dependent measures of e.g. escape latency (s), total path (cm) and total speed (cm.s-1). Treated groups received i.p. injection of QNB (2.0 mg.kg-1) in a volume of 1 mL.kg-1 1 h before the session followed 30 min later by the therapeutic dose of 6-chlortacrine (0.9, 1.8 or 3.6 mg.kg-1) or saline (non-treated QNB group). The decrease in the total path within the acquisition session in treated rats was compared to that of non-treated controls which received normal saline instead of cholinesterase inhibitor and blank controls which received saline instead of both, QNB and inhibitor. Data were log-transformed and subjected to factorial ANOVA, followed by Dunnett's test or Tukey's post-hoc. Saline-treated controls reduced their total path substantially from swim 1 to swim 10 ($F(9, 0.53) = 5.14$, $p < 0.001$) whereas no progression was found in group treated with QNB only ($F(9, 0.14) = 1.67$, $p = 0.11$). When the treatment with 6-chlortacrine was provided, significant improvement was evident in group treated with the dose of 1.8 mg.kg-1 ($p < 0.05$) representing brain cholinesterase inhibition of 49.0%. Other two tested doses were responsible for brain cholinesterase inhibition 39.0 and 68.5%, respectively and showed weaker effect. In general, novel cholinesterase inhibitor 6-

chlortacrine ameliorated QNB-induced cognitive impairment and will be further investigated, including detailed pharmacokinetic and behavioral studies.

Disclosures: **J. Misik:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GACR project P303/12/0611. **M. Hrabinova:** None. **J. Korabecny:** None. **E. Nepovimova:** None. **O. Soukup:** None. **K. Kuca:** None. **J. Kassa:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.09/C22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The National Natural Science Foundation of China(81300942)

Title: Isopimaric acid protects hippocampal neural injury and improves learning-memory deficits in APP/PS1 mice

Authors: ***L. WANG**[1], Z. LIU[2], J. JIA[3], Z. TANG[4], Y. SHUI[5], J. JIAO[6];
[1]China-Japan Friendship Hosp., Beijing, China; [2]Dept. of Neurology, China-Japan friendship hospital, Beijing, China; [3]Dept. of Physiology, Capital Med. Univ., Beijing, China; [4]Dept. Med. Genetics, Capital Med. Univ., Beijing, China; [5]Pain Mgmt. Center, China-Japan friendship hospital, Beijing, China; [6]Dept. of Neurology, China-Japan friendship hospital., Beijing, China

Abstract: Alzheimer disease is the most common and important neurodegenerative disease. The extreme atrophy of the hippocampus is the most prominent finding in pathology. The present experiment investigated the protective effect of isopimaric acid (ISO), the BK_{Ca} channel activator, on hippocampal neural injury in 4-month-old APP/PS1 mice. In the Morris water maze, the probe test demonstrated an improved spatial memory after chronic injection of ISO into the lateral ventricle. We did electrophysiological experiments using hippocampal slices of mice after behavior. Field excitatory postsynaptic potential (fEPSP) was recorded in CA1 region of hippocampus. Compared with WT group, the paired-pulse facilitation (PPF) was significantly decrease and long-term potentiation (LTP) was suppressed in APP/PS1 mice. Both PPF and LTP of hippocampus were recovered by ISO treatment. Whole-cell patch-clamp recordings were performed to assess the excitability of hippocampal pyramidal cells by calculating the frequency of evoked spikes and BK_{Ca} channel activity by measuring the half-width of evoked action potentials. In contrast to WT group, activity of the BK_{Ca} channels was suppressed and excitability of hippocampal neurons was elevated in the APP/PS1 group. The recovery of BK_{Ca} channel activity accompanied reduction of neuronal excitability in hippocampal pyramidal cells

by ISO treatment. The present findings suggest that ISO can normalize hippocampal neural injury and improve learning-memory deficits in AD.

Disclosures: **L. Wang:** None. **Z. Liu:** None. **J. Jia:** None. **Z. Tang:** None. **Y. Shui:** None. **J. Jiao:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.10/C23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The General Insurance Association of Japan

Title: Deficits in Morris water maze performance exacerbated by minimal neocortical injury in a mouse model of Alzheimer's disease

Authors: ***N. KATO**, J. ZOU;
Dept Physiol, Kanazawa Med. Univ., Ishikawa, Japan

Abstract: Brain injury is known as a major risk factor for Alzheimer's disease (AD). If injury limited to the neocortex induced deficits in hippocampus-dependent learning, remote effects would have to be involved, which might influence the hippocampal function electrophysiologically and biochemically. This possibility was tested by using Morris water maze test (MWM) twice. Wild-type and 3xTg-AD model mice were subjected to the first Morris water maze test. Based on the MWM performance, each type of mice was grouped into two, with the average MWM score esenssially the same between the two. Then, a tiny amount of glutamate (0.1 M, 0.2 μl) as neurotoxin, or the same amount of saline as vehicle control, was injected into the parietal cortex, thus creating 4 experimental groups. After a 2-week survival, the second MWM was performed, with a different goal location. The MWM score was normal and did not show difference between the glutamate and saline groups in wild-type mice, whereas glutamate-treated 3xTg mice performed the test worse than controls. After behavior, hippocampal tissue removed from the 4 groups was subjected to DNA microarray analysis with Mouse Gene 1.0 ST (Affimetrix). Glutamate-induced lesion significantly changed expression of two genes more than 1.5 fold in wild-type mice only, and expression of 7 other genes in 3xTg only. Synaptic transmission in the hippocampal CA1 synapses was more efficient in the 3xTg glutamate group than in the others. Three mice from each group were used to assess the size of parietal cortex lesion histologically. These results suggest that even a small injury restricted to the parietal cortex, which failed to induce learning deficits in wild-type mice, may remotely affect the hippocampal function in 3xTg mice, changing the profile of gene expression and the strength of synaptic transmission.

Disclosures: **N. Kato:** None. **J. Zou:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.11/C24

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Low-dose ketamine, spine density and cognition in a mouse model of Alzheimer's disease

Authors: J. SMITH, P. N. PALLIER, A. T. MICHAEL-TITUS, *G. J. MICHAEL;
Barts & London SMD, London, United Kingdom

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that affects millions worldwide, but currently treatment options for this condition are very limited. Synaptic loss is a major pathophysiological feature of AD that correlates with disease severity. It has been shown that low-dose ketamine has antidepressant properties and induces synaptogenesis and dendritic spine growth. As AD is associated with major synaptic changes, we hypothesised that low-dose ketamine could reverse the synaptic loss seen in AD, and thereby improve cognition. The study was carried out in the TASTPM murine model of AD. Mice (6-7 months old) were tested in the open field (OF), the novel object recognition (NOR), and the Morris water maze (MWM) tests. TASTPM mice and matched wild-type C57BL/6J mice received either ketamine (10 mg / kg) or 0.9% saline i.p. (n=6). The ketamine dose was based on previous studies that had shown antidepressant and pro-synaptogenesis effects at this dose. All injections were performed a minimum of 4 hours before the start of behavioural testing. All mice were tested in the OF, and then in the NOR test, followed by the MWM test. Each mouse received three repeated injections of saline or ketamine spaced over two weeks. TASTPM mice were clearly hypoactive in the OF, and this was not modified significantly by ketamine. Ketamine treatment induced anxiolytic-like effects in wild-type mice, as shown by an increased time spent in the centre zone of the OF, but this effect was not seen in TASTPM mice. No behavioural deficit was found in the NOR test or in the acquisition phase or probe trial of the MWM test in TASTPM mice, compared to wild-type mice. Ketamine led to a moderate and non-significant improvement in performance in both wild-type and TASTPM mice in the probe trial of the MWM test. Spine density was assessed using Golgi-Cox staining in the hippocampus CA1 region; no differences were found between genotypes or drug treatment groups. Overall, the results do not support the hypothesis that low-dose ketamine exerts beneficial effects in this mouse model of AD; however, the TASTPM mice, unexpectedly, had very limited alterations in behaviour compared to wild-type mice. Future studies investigating whether, in this murine model of AD, ketamine can improve the histopathology and the behavioural deficits at a later stage of the disease, when basal impairment in performance is much clearer, are warranted.

Disclosures: **J. Smith:** None. **P.N. Pallier:** None. **A.T. Michael-Titus:** None. **G.J. Michael:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.12/C25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FORUM Pharmaceuticals Inc.

Title: Activation of α7 nACh receptors in Aβ overproducing transgenic mice augments hippocampal theta oscillation

Authors: ***M. STOILJKOVIC**[1], D. NAGY[1], G. P. HAJOS[1], C. KELLEY[1], G. KOENIG[2], T. PISER[2], L. LEVENTHAL[2], M. HAJÓS[1];
[1]Comparative Med., Yale Univ. Sch. of Med., New Haven, CT; [2]FORUM Pharmaceuticals Inc., Watertown, MA

Abstract: Selective activation of α7 nicotinic acetylcholine receptors (α7 nAChRs) is considered as a potential new symptomatic treatment strategy for cognitive deficits in Alzheimer's disease (AD). In fact, exploratory and clinical Phase II trials suggest that α7 nAChR agonists may improve cognitive function in AD patients. However, a multifaceted interaction between amyloid ß (Aβ) and α7 nAChRs has been shown, indicating that pharmacological effects of α7 nAChR agonists in AD patients might be modified by high level Aβ proteins. Therefore, in the present study efficacy of a recently characterized α7 nAChR agonist FRM-17874 was evaluated in 8-month old Aβ overproducing APP/PS1 transgenic (APP/PS1), and compared to that in age-matched wild-type mice. Hippocampal theta oscillation was elicited by stimulation of the brainstem nucleus pontis oralis (nPO) in APP/PS1 and WT mice under urethane anesthesia. In line with our earlier findings, TG mice showed reduced hippocampal theta power in response to nPO stimulation (Scott et al., 2012), as well as significantly reduced gamma power and theta phase - gamma amplitude coupling (expressed as Modulation Index). Previous studies have shown that α7 nAChR agonists, including FRM-17874 augment theta oscillation in the hippocampus CA1 region (Siok et al., 2006), which is considered as a possible mechanism contributing to their pre-cognitive effects. FRM-17874, at an already established effective dose (3 mg/kg, sc) significantly augmented power of elicited hippocampal theta oscillation in both APP/PS1 and WT mice ($F(3,28) = 9.913$, $p = 0.0005$). Furthermore, compared with their saline-treated respective controls, FRM-17874 caused a significant increase in theta power in both groups of mice showing identical efficacy, but did not affect the peak theta frequency in either group. The magnitude of drug effect in 8 months old WT and TG animals was comparable to previously observed effects in 3 months old mice and rats as well as with effects of current AD

drugs donepezil and memantine tested using the same assay. FRM-17874 did not change power of elicited gamma (30-90 Hz) power in WT or APP/PS1 mice; however it increased theta phase frequency (4-7 Hz) and higher frequency (75-95 Hz) gamma band amplitude coupling in WT mice. These results indicate that high levels of amyloid ß peptides do not interfere with ability of the pharmacology of α7 nAChR agonists to augment power of hippocampal theta oscillation, and provide further support for targeting α7 nAChRs as potential AD pharmacotherapy.

Disclosures: **M. Stoiljkovic:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; FORUM Pharmaceuticals Inc., Watertown, MA. **D. Nagy:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; FORUM Pharmaceuticals Inc., Watertown, MA. **G.P. Hajos:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; FORUM Pharmaceuticals Inc., Watertown, MA. **C. Kelley:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; FORUM Pharmaceuticals Inc., Watertown, MA. **G. Koenig:** A. Employment/Salary (full or part-time):; FORUM Pharmaceuticals Inc., Watertown, MA. **T. Piser:** A. Employment/Salary (full or part-time):; FORUM Pharmaceuticals Inc., Watertown, MA. **L. Leventhal:** A. Employment/Salary (full or part-time):; FORUM Pharmaceuticals Inc., Watertown, MA. **M. Hajós:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; FORUM Pharmaceuticals Inc., Watertown, MA.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.13/C26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG044712

AG014449

P30 AG028383

St. Mary's Foundation

Title: The role of the locus coeruleus projection system in neuronal and cerebrovascular dysfunction in preclinical AD

Authors: *S. C. KELLY[1,2], E. J. MUFSON[4], P. T. NELSON[5], S. E. COUNTS[1,3,6]; [1]Translational Sci. and Mol. Med., [2]Cell and Mol. Biol. Program, [3]Dept. of Family Medecine, Michigan State Univ., Grand Rapids, MI; [4]Neurobio., Barrow Neurolog. Inst., Pheonix, AZ; [5]Pathology and Lab. Med., Univ. of Kentucky, Lexington, KY; [6]Haunstein Neurosci. Inst., Mercy Hlth. St. Mary's, Grand Rapids, MI

Abstract: Noradrenergic locus coeruleus (LC) neuron loss is a major feature of Alzheimer's disease (AD). The LC is the primary source of norepinephrine (NE) in the brain, where it modulates attention, memory, and arousal in vulnerable cognitive regions such as prefrontal cortex and hippocampus. Previously, we demonstrated a ~35% loss of LC neurons in postmortem tissue of subjects who died with an antemortem clinical diagnosis of amnestic mild cognitive impairment (aMCI, a prodromal AD stage) compared to subjects who died with no cognitive impairment (NCI) ($p<0.01$) obtained from the Rush Religious Orders Study. However, the extent to which LC neurodegeneration occurs in preclinical, pre-MCI stages of AD is unclear. Furthermore, LC-mediated NE signaling has been shown to play a role in cerebrovascular (CV) health, including blood brain barrier maintenance and neurovascular coupling, suggesting that LC degeneration may impact the high comorbidity of CV disease and AD. However, the effect of LC degeneration on CV function during disease onset is also unclear. To begin to address these issues, we analyzed LC tissue obtained postmortem from University of Kentucky AD Center subjects classified antemortem as NCI but who displayed varying levels of Braak stage neuropathology at autopsy. In particular, NCI subjects who died with Braak stages III-V are believed to have been in the preclinical stages of AD. In preliminary studies, LC tissue from NCI-Braak 0, NCI-Braak II, and NCI-Braak IV (n = 5/group) was immunostained with tyrosine hydroxylase (TH, a marker for NE synthesis) and analyzed using unbiased stereology to estimate total LC neuron number (neuromelanin-containing LC neurons +/- TH) and the percentage of TH+ LC neurons. Preliminary analysis revealed a ~18% loss of LC neurons in NCI-Braak IV (e.g., preclinical AD) cases compared to NCI-Braak 0-II cases. Furthermore, we found a ~15-20% loss of TH+ LC neurons in NCI-Braak IV compared to NCI-Braak 0-II cases. Pilot studies also revealed a substantial increase in the presence of pathological markers (e.g., tau AT8, TDP-43, 8-OHG) in LC neurons in NCI-Braak IV compared to NCI-Braak 0-II. Hence, LC neurodegeneration appears to mark the preclinical stages of AD prior to the onset of MCI. To begin to understand the relationship between LC neuropathology in preclinical AD and CV function, morphometric data will be correlated with postmortem CV variables (e.g., microinfarcts). Taken together, these data should give us a better understanding of how multifactorial noradrenergic pathways contribute to neuronal and vascular pathologies during the onset of AD.

Disclosures: **S.C. Kelly:** None. **E.J. Mufson:** None. **P.T. Nelson:** None. **S.E. Counts:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.14/C27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH-NIA grant R21AG029318

VA merit review

Title: Somatostatin receptor subtype-4 agonist enhances learning and memory in aged SAMP8 mice

Authors: *K. A. WITT[1], A. M. CRIDER[2], J. E. MORLEY[3], S. A. FARR[3], K. E. SANDOVAL[2]; [1]Pharmaceut. Sci., Southern Illinois Univ. Edwardsville, Edwardsville, IL; [2]Pharmaceut. Sci., Southern Illinois Univ. Edwardsville Sch. of Pharm., Edwardsville, IL; [3]Intrnl. Med., St. Louis Univ. Sch. of Med., Saint Louis, MO

Abstract: Use of a selective somatostatin receptor subtype-4 (SSTR4) agonist has been identified as a potential means to mitigate the cognitive decline in pathologies such as Alzheimer's Disease (AD), via enhancement of neprilysin activity and/or direct receptor action within the neocortex and hippocampus. Herein we evaluated the somatostatin receptor SSTR4 agonist NNC 26-9100 effects using the senescence accelerated mouse prone-8 (SAMP8) model of cognitive decline at pre-pathology (4-month), early-pathology (12-month), and later-pathology (15-month) stages of development. Mice were chronically treated with NNC 26-9100 (10 mg/kg, i.p. daily for 28 days) or matched vehicle. NNC 26-9100 treatment reduced acquisition learning (T-maze, day-21) in 4-month mice, while enhancing learning in 12-month mice, and showing no change in 15-month mice when compared to their respective age-matched vehicle treated controls. In 4-month mice NNC 26-9100 did not impact memory retention (T-maze, day-28); however, it enhanced memory in both 12- and 15-month mice when compared to respective age-matched vehicle treated controls. Cortical tissues were extracted for neprilysin activity and Western blot analysis. NNC 26-9100 treatment did not increase neprilysin activity in 4-month mice, but increased activity in both 12- and 15-month mice compared to vehicle. Amyloid precursor protein and SSTR4 expression levels did not change, while neprilysin expression increased in 15-month mice with NNC 26-9100 treatment, compared to vehicle. These findings suggest the use of a selective SSTR4 agonist may mitigate the cognitive decline in aged pathologic mice, but not in pre-pathologic mice.

Disclosures: K.A. Witt: None. **A.M. Crider:** None. **J.E. Morley:** None. **S.A. Farr:** None. **K.E. Sandoval:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.15/C28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This work was supported by Major Scientific and Technological Special Project for "Significant New Drugs Creation" (No. 2009ZX09302-003), National Natural Science Foundation of China (No. 81102830, 81073120).

Title: Quercetin protects against the Aβ-induced amnesic injury through inhibiting RAGE-mediated pathway and preserving the neurovascular unit

Authors: *R. LIU, D. ZHOU, X. BAI, C. HUANG, J. SONG, G. DU;
Inst. of Materia Medica, Chinese Acad. of Me, Beijing, China

Abstract: Quercetin is one of the most common flavonoids existing widely in many edible plants. It has demonstrated protective effects against Aβ-induced toxicity on both neurons and endothelial cells. However, whether or not quercetin has an effect on the neurovascular coupling is unclear. In the present study, we aim to investigate the anti-amnesic effects of quercetin and to explore the underlying mechanisms. The aggregated Aβ25-35 was injected into the right lateral ventricle in mice. Quercetin was administrated orally for 8 days after injection. Learning and memory behaviors were evaluated by measuring spontaneous alternation in Morris Water Maze test and the step-through positive avoidance test. The regional cerebral blood flow was monitored before the Aβ25-35 injection and on seven consecutive days after injection. Mice were sacrificed and cerebral cortices were isolated on the last day. The effects of quercetin on the neurovascular unit (NVU) integrity, microvascular function and cholinergic neuronal changes, and the modification of signaling pathways were tested. Our results demonstrate that quercetin treatment for Aβ25-35-induced amnesic mice improved the learning and memory capabilities and conferred robust neurovascular coupling protection. In these effects, quercetin improved the spatial learning and memory effectively across the acquisition training period and the memory capability trials in behavioral tests. Furthermore, quercetin maintained the integrity of NVU major components, reduced the level of neurovascular oxidation, modulated the rCBF value of cerebral microvessels, improved the function of cholinergic system, and regulated the neurovascular RAGE signaling and ERK/CREB/BDNF pathways. In conclusion, in Aβ25-35-induced amnesic mice, optimal doses of quercetin administration were beneficial. Quercetin protected the NVU through reduction of oxidative damage, inactivation of RAGE-mediated pathway and preservation of cholinergic neurons, offering an alternative medication for Alzheimer's disease.

Disclosures: **R. Liu:** None. **D. Zhou:** None. **X. Bai:** None. **C. Huang:** None. **J. Song:** None. **G. Du:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.16/C29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT 11090091

FONDECYT 1130747

MECESUP UCO1311

Title: Changes in the purinergic and glutamatergic neurotransmission in a model of Alzheimer's disease modulated by P2X receptors

Authors: *F. SÁEZ-ORELLANA, P. A. GODOY, T. SILVA-GRECCHI, K. M. BARRA, J. FUENTEALBA;
Univ. De Concepción, Concepción, Chile

Abstract: Currently there are around 24 million cases of dementia worldwide, the most common cause of dementia is Alzheimer´s Disease (AD) affecting mainly elderly people. The etiology of AD is still unknown, but it is believed that the main culprits are the soluble oligomers of Amyloid beta (Aβ) peptide. In literature is reported that Aβ increases the expression of P2X7 *in vitro* and *in vivo*, and also has been demonstrated that there are changes in the levels of GluN2A and GluN2B. Another effect of Aβ is a diminishment of AMPAR at synaptic level, which explains the memory loss. The aim of this work was to study the effects of acute (1h) and chronic (24h) exposition to Aβ (0.5 μM) on the glutamatergic, GABAergic and purinergic transmission. Acute Aβ incubation induced an increase in the amplitude and frequency of mEPSC in primary hippocampal cultures (Control: 100±15%, 14±0.2 pA; Aβ: 248±33%, 19±0.3), which was prevented by the non-selective P2XR antagonist PPADS (10μM) and also by ATP hydrolysis with apyrase (3U/ml) (Aβ+PPADS: 111 ±18%, 11 ±0,4pA; Aβ+apyrase: 99 ±17%, 14 ±0.6pA); besides, we observed an acute increased insertion of AMPAR that could explain the increased amplitude of mEPSC. On mIPSC we observed a non-significant reduction of frequency and a significant diminishment in amplitude (control: 100 ±19%, 19.3 ±0.7 pA; Aβ: 70 ±11%, 14.7 ±0.9 pA). In chronic treatment we observed that Aβ induced a decrease in the frequency and amplitude of mEPSC in primary hippocampal cultures (control: 100±20%, 9.7±0.2pA; Aβ: 28±7%, 6.3±0.1pA); and interestingly, we found an increase in the amplitude of ATP-evoked currents that is related to an increased expression of P2X1, 2 and 7; moreover, we observed a right shift in the dose-response curve to ATP analyzed by Ca2+ microfluorimetry. In this model we also evaluated the effects of Aβ in glutamatergic components and found a decrease in the levels of both NMDAR and AMPAR which can explain the decrease in amplitude of mEPSC, in line with these findings we found a decreased expression of PSD95; interestingly, all the changes induced by Aβ were prevented by the inhibition of P2XR by PPADS or apyrase. In conclusion we can suggest that soluble oligomers of Aβ can disrupt the

main components of excitatory and inhibitory synaptic transmission at pre- and post-synaptic level, and also change the expression and function of purinergic neuromodulation; this can be a new target for the treatment of this pathology and others diseases characterized by increased purinergic transmission.

Disclosures: **F. Sáez-Orellana:** None. **P.A. Godoy:** None. **T. Silva-Grecchi:** None. **K.M. Barra:** None. **J. Fuentealba:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.17/C30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG2161201

Title: Impact of 17-beta estradiol (E2) loss on hippocampal synaptic function in an Alzheimer's disease rat model and the role of GluN2B-NMDARs

Authors: ***L. A. SMITH**[1], T. TOWN[2], L. L. MCMAHON[1];
[1]Cell, Developmental, and Integrative Biol., Univ. of Alabama, Birmingham (UAB), Birmingham, AL; [2]Physiol. & Biophysics, Zilkha Neurogenetic Inst., Los Angeles, CA

Abstract: Alzheimer's disease (AD) is the most common form of dementia for those 65 years and older, and interestingly women account for over two-thirds of those affected. Current treatments only target symptoms of the disease. Therefore, there is great need for disease-modifying therapies that can slow or halt disease progression before debilitating symptoms arise. Human imaging data and rodent studies show that pre-symptomatic alterations in synaptic efficacy are correlated with increased soluble protein aggregates of amyloid beta (Aβ) and hyperphosphorylated tau (p-tau), and that these soluble aggregates are more toxic to synaptic function than when they deposit as characteristic lesions in the form of amyloid plaques and tau tangles. Soluble Aβ and tau inhibit N-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) possibly through excessive activation of extrasynaptic GluN2B-containing NMDARs. Previous work in our lab has shown that 17β-estradiol (E2) treatment, the primary ovarian estrogen, increases hippocampal CA1 pyramidal cell spine density, synaptic GluN2B-mediated NMDAR current, LTP magnitude, and learning in surgically menopausal female rats. Here we investigate early synaptic alterations prior to plaque and tangle formation in the novel comprehensive TgF344-AD (Tg) rat model to test the hypothesis that E2 confers its neuroprotective effects in AD at the synapse by enhancing synaptic and decreasing extrasynaptic currents mediated by GluN2B-containing NMDARs. Current data show that at 3 and 6 months of age there is no deficit in excitatory transmission at CA3-CA1 synapses in both males and acutely

ovariectomized (OVX) females. Presynaptic function and AMPA receptor mediated transmission are also unaltered. Additionally, there is no change in the magnitude of LTP in the presence of the GluN2B-subunit selective antagonist Ro25-6981 (RO) in either sex. Interestingly, there is a trend towards increased block of NMDARs by Ro25-6981 in Tg hippocampal slices from OVX females during the tetanus used to induce LTP, possibly suggesting increased activation of extrasynaptic GluN2B-containing NMDARs, consistent with current literature in AD mouse models. On-going studies are characterizing excitatory transmission, contribution of GluN2B-containing NMDARs, synaptic plasticity, and effects of loss and replacement of E2 in OVX female WT and Tg rats on hippocampal synaptic function over the lifespan. Understanding the underlying mechanism of how E2 is neuroprotective at the synapse throughout early AD pathology can inform future design of AD-modifying therapeutics.

Disclosures: L.A. Smith: None. **T. Town:** None. **L.L. McMahon:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.18/C31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: France Alzheimer

Title: Early LTP deficits in 3xTg mice are restored by D-serine

Authors: *J. VERAN[1], A. PANATIER[1], G. BONVENTO[2], S. H. R. OLIET[1];
[1]Neurocentre Magendie, Bordeaux, France; [2]Sci. du Vivant, CEA, Fontenay aux roses, France

Abstract: At early stages of Alzheimer's disease (AD), intracellular accumulation of Aβ leads to cognitive and synaptic transmission impairment. Such deficits are reproduced in the triple transgenic model 3xTG mice in which basal excitatory synaptic transmission and long-term potentiation (LTP) in the hippocampus are significantly impaired, long before plaque deposits. LTP induction at CA3-CA1 synapses depends on NMDA receptors (NMDARs), and we have shown previously that these receptors were gated by glial D-serine. We here investigated whether the synaptic plasticity deficits observed in 3xTg mice at early age could be due to a reduced level of occupancy of NMDAR glycine-binding sites, on which D-serine is binding. To this end we recorded field excitatory postsynaptic potentials (fEPSPs) at CA3-CA1 synapses in acute hippocampal slices obtained from 6-7 months old female mice. As previously described, both input/output relationship and LTP were impaired significantly. We then assessed the level of occupancy of NMDAR co-agonist binding site by applying exogenous D-serine in the bath. The increase in NMDAR-mediated fEPSPs induced by D-serine was larger in 3xTG mice, indicating that the level of occupancy of NMDAR co-agonist site is lower in these animals. Most

importantly, exogenous D-serine rescued completely LTP induction in the 3xTG mice. Altogether, these results suggest that early deficits in AD are associated with a decrease in D-serine in the synaptic cleft.

Disclosures: J. Veran: None. **A. Panatier:** None. **G. Bonvento:** None. **S.H.R. Oliet:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.19/C32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NWO

Title: AMPA-receptor subunit composition determines the susceptibility of synapses and memory to amyloid-beta

Authors: *N. R. REINDERS[1], Y. PAO[2], M. C. RENNER[1], R. MALINOW[2], H. W. KESSELS[1]; [1]Netherlands Inst. of Neurosci., Utrecht, Netherlands; [2]Univ. of California, San Diego, CA

Abstract: Amyloid-β (Aβ) is a prime suspect to cause Alzheimer's disease (AD). Soluble oligomeric clusters of Aβ trigger synaptic depression by reducing AMPA- and NMDA-type glutamate receptor currents, and initiate memory impairment in mouse models for AD. We here show that these Aβ-driven effects depend on AMPA-receptor subunit composition. Hippocampal neurons were resistant against Aβ-mediated spine loss and synaptic depression of both AMPA-receptor and NMDA-receptor currents when they express AMPA-receptor subunits GluA1 and GluA2 but lack the GluA3 subunit. In addition, an Aβ-mediated blockade of LTP was seen in hippocampal slices only when they express GluA3. Furthermore, while APP/PS1-transgenic mice showed significant impairment in contextual fear memories at 6 months of age, fear memories in littermate GluA3-deficient APP/PS1 mice remained unaffected. These experiments suggest that Aβ selectively disrupts synapses that are enriched for GluA3.

Disclosures: N.R. Reinders: None. **Y. Pao:** None. **M.C. Renner:** None. **R. Malinow:** None. **H.W. Kessels:** None.

Poster

299. Neuropharmacology and Neurotransmission in Dementia

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 299.20/C33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG042475

NIH AG027219

NIH AG028383

NIH AG014449

Alz Asso NIRG-11-198378

Title: Cell signaling proteins in the early phase of Alzheimer's disease

Authors: *M. A. ANSARI[1], E. J. MUFSON[2], S. W. SCHEFF[3];
[1]Sanders-Brown Ctr. on Aging, Univ. Kentucky, Lexington, KY; [2]Dept. of Neurobio., Barrow Neurol. Inst., Phoenix, AZ; [3]Ctr. on Aging, Univ. of Kentucky, Lexington, KY

Abstract: Alzheimer's disease (AD) is the most common form of age-related dementia. Multiple factors are involved in the disease progression and the etiology of AD is still not well understood. A serine/threonine kinase, glycogen synthase kinase-3 (GSK-3), is an important kinase involved in normal synaptic function. Recent studies have linked two isoforms (GSK-3α and GSK-3β) to histopathological hallmarks of AD. Another serine/threonine and tyrosine kinase, Lin-11- Isl-1-Mec-3 kinase (LIMK-1/2), is believed to participate in various cellular functions including synapse formation and also may play an important role in the progression of AD pathology. This study evaluated the changes in both enzymes in the hippocampus, one of the earliest brain region affected in the progression of AD. Our laboratory has previously shown a significant loss of synapses in the hippocampus in subjects with amnestic mild cognitive impairment (aMCI). The present study tested whether or not changes in these cell signaling proteins occur in a pre-aMCI phase of AD. We analyzed short post-mortem hippocampal tissues obtained from three different cohorts: (1) individuals with low or no AD-type pathology and no cognitive impairment (LP-NCI), (2) individuals with high AD-type pathology and no cognitive impairment (HP-NCI), and (3) individuals with aMCI. Levels of phosphorylated and non-phosphorylated forms of both GSK-3α/β and LIMK-1/2 were assessed in all subjects. Individuals with aMCI manifested a significant increase in phosphorylation of GSK3β compared to both age- and postmortem-matched NCI cohorts. Levels of GSK-3α and LIMK-1/2, both phosphorylated and non-phosphorylated form, remained unchanged across all groups. These results suggest that altered cell signaling kinases (i.e. GSK-3β) are possibly driving synaptic dysfunction and cognitive loss in aMCI, which is believed to be an early stage of AD.

Disclosures: M.A. Ansari: None. **E.J. Mufson:** None. **S.W. Scheff:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.01/C34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF BCS-1316829

NIH NS-42867

NIH NS-73134

NIH AG017802

NIH AG014308

Sigma Xi Grants-in-Aid

Kent State University Research Council

Title: Alzheimer's disease pathology in aged chimpanzees

Authors: *M. K. EDLER[1], P. R. HOF[3], E. J. MUFSON[4], W. D. HOPKINS[5,6], J. J. ELY[7], S. E. PEREZ[8], J. M. ERWIN[9], C. C. SHERWOOD[9], M. A. RAGHANTI[1,2];
[1]Sch. of Biomed. Sci., [2]Dept. of Anthrop., Kent State Univ., Kent, OH; [3]Fishberg Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Departments of Neurobio. and Neurol., Barrow Neurolog. Inst., Phoenix, AZ; [5]Div. of Developmental and Cognitive Neurosci., Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [6]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [7]MAEBIOS-TM, Alamogordo, NM; [8]Dept. of Neurolog. Sci., Rush Univ., Chicago, IL; [9]Dept. of Anthrop., The George Washington Univ., Washington, DC

Abstract: Alzheimer's disease (AD) is considered a uniquely human disorder, characterized neuropathologically by beta-amyloid plaques (Aβ) and neurofibrillary tangles (NFTs). While amyloid plaques and tau pathology have been identified in species other than humans, these animals typically present with one type of pathology and lack the cognitive deficits typical of AD. Prior limited research on aged great apes demonstrated widespread Aβ pathology in absence of significant tauopathy. The objective of the current research was to evaluate AD pathology in a large group of aged chimpanzees (n = 20, ages 37-62 years). Immunohistochemical and stereologic techniques were used to map and quantify Aβ plaques (6E10, Aβ42), cerebrovascular amyloid, and tau pathology (AT8, CP13, MC1, PHF1) in postmortem brain samples from three regions affected by AD in humans (i.e., prefrontal cortex, middle temporal gyrus, and hippocampus). Two-thirds of the elderly chimpanzees exhibited at least one form of Aβ pathology (i.e., plaques and vascular), with Aβ-positive vasculature more common than plaques. Three of the oldest chimpanzees (ages 57-62 years) displayed congophilic cerebral amyloid angiopathy (CAA). At least one form of tau pathology (i.e., preNFTs, NFTs, and neuritic tau plaques) was found in all individuals. Overall, preNFTs and NFTs were most numerous in the hippocampus, while neuritic tau plaques were more prevalent in the prefrontal cortex. Two

chimpanzees had significant, potentially non-age related tauopathy. A 39-year-old male presented with NFTs in the prefrontal cortex, lack of nearly any tau pathology in the hippocampus, and absence of Aβ pathology in all three areas. Conversely, a 57-year-old male demonstrated extensive levels of all tau lesion types in each region, with the hippocampus most severely affected, as well as scarce Aβ plaques and CAA. The co-occurrence of Aβ and tau pathology in aged chimpanzees indicates these lesions are not specific to the human brain; however, further correlative analyses with cognitive and behavioral assessments are needed to shed light on whether neurodegenerative diseases, such as AD, are truly human-specific. Future investigations will characterize the composition of tau isoforms (three or four repeats) in preNFTs, NFTs, and tau plaques, examine the potential coexistence of neurodegenerative pathologies, such as Lewy bodies and synaptopathy, and study the association of DNA sequence polymorphisms in the amyloid precursor protein and tau genes with observed neuropathy in elderly chimpanzees.

Disclosures: **M.K. Edler:** None. **P.R. Hof:** None. **E.J. Mufson:** None. **W.D. Hopkins:** None. **J.J. Ely:** None. **S.E. Perez:** None. **J.M. Erwin:** None. **C.C. Sherwood:** None. **M.A. Raghanti:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.02/C35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Academy of Finland grant EuroEPINOMICS (AP)

The Sigrid Juselius Foundation (AP)

CIMO (Center for International Mobility) (DM)

Foundation for Polish Science grant MPD/2009/4 (KL)

National Science Center of Poland grant 2012/05/N/NZ/02500 (DM, KL)

Ministry of Science and Higher Education grant DNP/N119/ESF-EuroEPINOMICS/2012 (KL)

Title: Aggravated post-traumatic epileptogenesis associates with acquired channelopathy in the perilesional cortex of APP/PS1 mouse model of Alzheimer`s disease

Authors: *D. MISZCZUK[1,2], K. J. DęBSKI[1], H. TANILA[2], K. LUKASIUK[1], A. PITKANEN[2]; [1]Nencki Inst. of Exptl. Biol., Warsaw, Poland; [2]Dept. of Neurbiology, A. I. Virtanen Inst. for Mol. Sciences, Univ. of Eastern Finland, Kuopio, Finland

Abstract: We tested a hypothesis that traumatic brain injury (TBI) in amyloidogenic genetic background leads to aggravated epileptogenesis which is associated with the development of chronic acquired channelopathy. Traumatic brain injury was induced with controlled cortical impact in APP/PS1 mice (n=14) or in their wild type (Wt) littermates (n=17). Epileptogenesis was monitored with video-EEG, and the expression level of voltage-gated ion channel subunits was assessed 16 wk post-TBI using Affymetrix microarrays. Epileptogenesis was affected both by the genotype and TBI. In APP/PS1-TBI group 88% developed epilepsy, in the Wt-TBI group 11% (genotype effect $p<0.01$), and in the APP/PS1-sham group 50% (TBI effect $p<0.05$). The most pronounced effect of genotype and TBI on the expression of ion channel subunits was found in the perilesional cortex as compared to the ipsilateral thalamus or hippocampus. In sham-operated mice, AD-genotype associated with down-regulation of expression of *Kcna6*, *Scn9a* and *Scn4b* and up-regulation of *Scn4a* as compared to Wt mice ($p<0.01$). In mice with TBI, AD genotype associated with up-regulation of *Hcn4* and *Scn7a* and down-regulation of *Kcnv1, Kcnk4* and *Scn9a* as compared to APP/PS1-sham mice ($p<0.01$). Moreover, we found an injury effect as Wt-sTBI mice displayed a decreased expression of *Scn3a* and *Cacna2d1* as compared to Wt-sham group ($p<0.01$). Interaction analysis between the genotype and the injury revealed differences in the alteration in expression of *Kcnt1*, *Scn3a* and *Scn7a*. Interestingly, correlation analysis revealed that the higher the seizure frequency, the lower the cortical expression of *Scn9a* ($r=-0.82$, $p<0.001$), *Scn4b* ($r=-0.67$, $p<0.001$) and *Kcnk4* ($r=-0.64$, $p<0.001$). The present study provides the first comprehensive evidence of the possible contribution of multi-channelopathy on exacerbated post-traumatic epileptogenesis in amyloidogenic genetic background.

Disclosures: D. Miszczuk: None. **K.J. Dębski:** None. **H. Tanila:** None. **K. Lukasiuk:** None. **A. Pitkanen:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.03/C36

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The development and characterisation of a high-throughput assay for evaluation of neuronal excitability and synaptic function in neuronal models of Alzheimer’s disease

Authors: *J. K. VIRDEE, Y. SINHA, S. GLOVER, F. FERRERIA, M. O'NEILL, J. WOLAK, D. URSU;
Eli Lilly and Co., Windlesham, United Kingdom

Abstract: Alzheimer’s disease (AD) is the most common form of dementia, currently with over 850,000 people in the UK being diagnosed with AD, a number that is predicted to increase to

over 2 million by 2050 (statistics from UK Alzheimer's Society). The excessive accumulation of amyloid-beta (Aβ) peptide, in particular the oligomeric form, is believed to be the seminal factor in disease causation, with clear effects demonstrated on neuronal viability but with more subtle and sometime difficult to assess disruptions seen on neuronal synaptic function. Understanding the effects of Aβ on neuronal and synaptic function and how novel compounds may restore abnormal synaptic activity in AD is therefore an attractive approach to pursue for identifying novel therapeutic targets. The aim of this study was to establish and validate methodologies for i) generation and characterisation of Aβ oligomers ii) measure overall neuronal cytotoxicity and iii) development of a new assay for evaluating effects of Aβ oligomers on neuronal excitability and synaptic function. Here we describe an assay based on electrically evoked responses to evaluate both neuronal somatic activity as well as synaptic function in cultured neuronal networks in a multi-well plate format. The assay integrates the state of the art screening platform FLIPR® Tetra with customised hardware and software that allows optimum control of electrical stimulation and recording at single well level. The assay utilises a set of bipolar stimulating electrodes that can deliver well defined stimulation protocols (ie voltage, frequency, number of stimuli) to the cultured neurons. The neuronal excitability and synaptic transmission can be measured indirectly by changes in fluorescence of a calcium sensitive dye in regions of interest located either proximal or distal to the stimulation electrodes. The synaptic nature of the assay was validated by screening a number of compounds with known pharmacological profiles as modulators of synaptic activity, acting either at presynaptic terminals (calcium channels, metabotropic glutamate receptors), postsynaptic region (AMPA, NMDA and GABA receptors) or generally acting on neuronal excitability (Nav, Kv ion channels). By using Aβ oligomer preparations characterised by DLS and EM we are showing disruption of neuronal and synaptic activity in the above validated assay following either acute (hours) or chronic (days) application.

Disclosures: **J.K. Virdee:** None. **Y. Sinha:** None. **S. Glover:** None. **F. Ferreria:** None. **M. O'Neill:** None. **J. Wolak:** None. **D. Ursu:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.04/C37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Grants Council of Hong Kong SAR HKUST660810

Research Grants Council of Hong Kong SAR HKUST661111

Research Grants Council of Hong Kong SAR HKUST661013

National Key Basic Research Program of China 2013CB530900

Hong Kong Research Grants Council Theme-based Research Scheme T13-607/12R

SH Ho Foundation

Title: Understanding the melanocortin circuit in the mouse hippocampus

Authors: *Y. SHEN[1,2,3], M. TIAN[1,2,3], Y. ZHENG[1,2,3], A. K. Y. FU[1,2,3], N. IP[1,2,3];
[2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., [1]The Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Hippocampal synaptic plasticity, the modification of the strength and efficacy of synaptic communication among neurons, is a major cellular mechanism underlying learning and memory. Melanocortins, which are the pro-opiomelanocortin-derived peptides originally shown to regulate energy metabolism, have been implicated in learning and memory enhancement. We recently found that melanocortin 4 receptor (MC4R) plays an important role in the regulation of hippocampal synaptic plasticity. Long-term potentiation in the hippocampus was significantly enhanced by *in vivo* MC4R activation and attenuated by MC4R knockdown. To study the circuitry and mechanisms of melanocortins/MCRs involved in hippocampal synaptic plasticity, we mapped the circuitry of pro-opiomelanocortin/MCRs in the mouse hippocampus. We also confirmed hippocampal melanocortin secretion in response to neuronal activity. These findings help to elucidate the hippocampal circuitry involved in learning and memory as well as the functions of the melanocortin system in the human brain; they may also reveal a functional association between brain metabolism and cognition.

Disclosures: Y. Shen: None. **M. Tian:** None. **Y. Zheng:** None. **A.K.Y. Fu:** None. **N. Ip:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.05/C38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RGC of HKSAR HK660810

RGC of HKSAR HK661111

RGC of HKSAR HK661013

National Key Basic Research Program of China 2013CB530900

HKRGC Theme-based Research Scheme T13-607/12R

Innovation and Technology Fund for State Key Laboratory ITCPT/17-9

S.H. Ho Foundation

Title: Melanocortin 4 receptor signaling modulation ameliorates synaptic dysfunction in Alzheimer's disease models

Authors: *M. TIAN[1,2,3], Y. SHEN[1,2,3], E. Y. L. CHENG[1,2,3], A. K. Y. FU[1,2,3], N. Y. IP[1,2,3]; [1]Mol. Neurosci. Ctr. and State key Laborary, The Div. of Life Science,, N/A, China; [2]Mol. Neurosci. Ctr., The Hong Kong University of Science and Technology, China; [3]State Key Lab. of Mol. Neurosci., The Hong Kong University of Science and Technology, China

Abstract: Amyloid-beta (Aβ) plaque aggregation is the hallmark of Alzheimer's disease (AD); cognitive dysfunctions in the disease are believed to be due to soluble Aβ oligomers that interrupt synaptic plasticity. We previously demonstrated that the G-protein-coupled receptor melanocortin 4 receptor (MC4R), which regulates energy homeostasis, is involved in synaptic plasticity regulation in the mouse hippocampus. The age-related decrease of the expression of the MC4R endogenous agonist α-MSH regulates MC4R signaling in the brains of AD model mice (APP/PS1 mice). Therefore, the present study examined whether the modulation of MC4R signaling ameliorates the synaptic dysfunction or pathology of AD. The MC4R agonist D-Tyr MTII rescued the soluble Aβ oligomer-induced synaptic transmission deficit in cultured hippocampal neurons. Furthermore, treatment with D-Tyr MTII alleviated the Aβ-induced synaptic plasticity impairment (i.e., long-term potentiation) in acute hippocampal slices and APP/PS1 mice. Interestingly, MC4R knockdown in the APP/PS1 mouse hippocampus abolished the rescue effect of D-Tyr MTII, suggesting that MC4R activation is critical for restoring the synaptic functions in AD. Our findings collectively demonstrate that MC4R signaling activation can ameliorate the impaired synaptic plasticity in AD. Thus, MC4R signaling is a potential target for therapeutic interventions for AD.

Disclosures: **M. Tian:** None. **Y. Shen:** None. **E.Y.L. Cheng:** None. **A.K.Y. Fu:** None. **N.Y. Ip:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.06/C39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Grants Council of Hong Kong (HKUST660810)

Research Grants Council of Hong Kong (HKUST661111)

the National Key Basic Research Program of China (2013CB530900)

Hong Kong Research Grants Council Theme-based Research Scheme (T13-607/12R)

the SH Ho Foundation

Title: The EphA4 inhibitor rhynchophylline ameliorates disease pathology in a mouse model of Alzheimer's disease

Authors: ***W.-Y. FU**[1,2,3], B. BUTT[1,2,3], K.-W. HUNG[1,2,3], F. C. F. IP[1,2,3], A. K. Y. FU[1,2,3], N. Y. IP[1,2,3];
[1]Div. of Life Sci., [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Alzheimer's disease is a neurodegenerative disease characterized by cognitive dysfunction. We previously demonstrated an unanticipated role of the signaling of EphA4, a receptor tyrosine kinase, in mediating hippocampal synaptic dysfunction in Alzheimer's disease. Furthermore, we have identified a small molecule rhynchophylline that can effectively block EphA4-dependent signaling in neurons, rescuing the impaired long-term potentiation in the APP/PS1 mouse model of Alzheimer's disease. Here, we further demonstrated that rhynchophylline ameliorates the Alzheimer's disease-like pathology in APP/PS1 mice. Soluble Aβ content and the extent of amyloid plaque deposition were significantly reduced in the cortices of APP/PS1 mice orally administrated with rhynchophylline. Intriguingly, rhynchophylline also regulated the microgliosis in APP/PS1 mice, suggesting that the small molecule reduces neuroinflammation. Taken together, rhynchophylline may be developed as a therapeutic intervention for Alzheimer's disease.

Disclosures: W. Fu: None. **B. Butt:** None. **K. Hung:** None. **F.C.F. Ip:** None. **A.K.Y. Fu:** None. **N.Y. Ip:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.07/C40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research (CIHR) Postdoctoral Fellowship to Asad Jan

Title: Establishing the role of eukaryotic elongation factor-2 kinase (eEF2K) in Alzheimer's disease and relevance for therapies

Authors: ***A. JAN**[1], G. LEPRIVIER[1], S. SOMASEKHARAN[1], M. VANDAL[2], F. CALON[2], M. HAYDEN[3], P. SORENSEN[1];
[1]BC Cancer Agency, Vancouver, BC, Canada; [2]Univ. of Laval, Quebec, QC, Canada; [3]Ctr. for Mol. Med. and Therapeut. (CMMT), Vancouver, BC, Canada

Abstract: Defects in neuronal energy metabolism and synaptic plasticity are thought to underlie cognitive dysfunction and neuronal loss in Alzheimer's disease (AD), and potentially other neurological diseases. The AMP-responsive protein kinase (AMPK), and its downstream target the eukaryotic elongation factor-2 kinase (eEF2K) couple neuronal energy metabolism to neural activity. This mechanism allows for tight regulation of dendritic mRNA translation in register with synaptic activity, and plays a pivotal role in modulating synaptic strength and plasticity. Previously, we have shown that tumor cells exploit the AMPK-eEF2K axis in developing resistance to nutrient deprivation (Leprivier, G. et al, Cell, 2013), and targeting this pathway represents a potential treatment strategy in cancers. Circumstantial evidence suggests that aberrant activation of the AMPK-eEF2K pathway may also be relevant to neurodegeneration in AD. Recent studies have demonstrated that eEF2K activity, measured by the phosphorylation of its substrate eEF2, is enhanced in AD brains. We have carried out detailed analysis in two AD transgenic mouse models (APPPS1 and 3x-Tg AD), and utilized pharmacological and gene silencing approaches in primary neuronal cultures to understand the relevance of AMPK-eEF2K pathway activity on amyloid-β induced deficits in dendritogenesis and oxidative stress. Our results suggest that the AMPK-eEF2K axis may provide a link between aberrant synaptic activity and neurodegeneration in AD, and represents a novel target for developing mechanism based therapies in AD and related dementias.

Disclosures: **A. Jan:** None. **G. Leprivier:** None. **S. Somasekharan:** None. **M. Vandal:** None. **F. Calon:** None. **M. Hayden:** None. **P. Sorensen:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.08/C41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

CNPq

FAPERJ

CAPES

Human Frontier Science Program (HFSP)

John Simon Guggenheim Foundation

Title: Liraglutide protects the brains of macaques against synapse loss caused by Abeta oligomers

Authors: *A. F. BATISTA[1], L. FORNY-GERMANO[1], N. M. LYRA E SILVA[1], J. BRITO-MOREIRA[1], M. GRALLE[1], S. BOEHNKE[2], B. COE[2], A. LABLANS[2], C. HOLSCHER[3], S. MARQUES[1], A. BLANCO MARTINEZ[1], W. KLEIN[4], J.-C. HOUZEL[1], S. FERREIRA[1], D. MUNOZ[2], F. DE FELICE[1];
[1]Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Queen's Univ., Kingston, ON, Canada; [3]Lancaster Univ., Lancaster, United Kingdom; [4]Northwestern Univ., Evanston, IL

Abstract: Alzheimer's disease (AD) is a debilitating neurodegenerative disorder and a major health problem worldwide. The lack of effective drugs to treat AD stimulates an intense pursuit of disease-modifying therapeutics. However, a major impediment to progress may lie in fundamental differences between humans and animal model species, largely rodents. Disruption of hippocampal insulin signaling has recently been described in the brains of AD patients and animal models of disease, which could contribute to cognitive impairment in this disease. Therefore, drugs that can restore normal insulin function in the central nervous system have been recently suggested as a promising novel approach to treat AD. Here, we evaluated the neuroprotective effects of liraglutide in cynomolgus macaques (Macaca fascicularis) that received intracerebroventricular injections of Aβ oligomers (AβOs). Liraglutide is an anti-diabetic agent that activates pathways common to insulin signaling through stimulation of glucagon-like peptide 1 (GLP-1) receptors. Nine female cynomolgus macaques were used. Three of them were sham-operated and served as controls. We performed intracerebroventricular (i.c.v.) injections of AβOs into six cynomolgus macaques. Two of these monkeys had been pre-treated with daily i.p. injections of liraglutide. Liraglutide administration continued daily until the last injection of oligomers. Brain sections were used for immunohistochemystry to evaluate the levels of synaptic markers. We found that AβOs induced a decrease in synapse number in the primate brain and reduced the levels of NMDA (GluN1 and GluN2B subunits), AMPA (GluA1 and GluA2 subunits) and insulin receptors. Liraglutide attenuated the impact of AβOs on synapses and on plasticity related receptors. These results establish the protective actions of liraglutide in the primate brain and indicates that a primate model of AD may be valuable not only for studying mechanisms responsible for AβOs toxicity, but also for exploring and evaluating new preventive therapeutic strategies for AD.

Disclosures: A.F. Batista: None. **L. Forny-Germano:** None. **N.M. Lyra e Silva:** None. **J. Brito-Moreira:** None. **M. Gralle:** None. **S. Boehnke:** None. **B. Coe:** None. **A. Lablans:** None. **C. Holscher:** None. **S. Marques:** None. **A. Blanco Martinez:** None. **W. Klein:** None. **J. Houzel:** None. **S. Ferreira:** None. **D. Munoz:** None. **F. De Felice:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.09/C42

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5F32AG043301-03

NS062413 (LME)

Title: Enhanced acetylation of tau in Alzheimer's disease causes deficits in synaptic plasticity and memory formation

Authors: *T. E. TRACY[1,2], P. SOHN[1,2], S. MINAMI[1,2], C. WANG[1,2], Y. ZHOU[1], D. LE[1], Y. LI[1], I. LO[1], R. PONNUSAMY[1], B. W. GIBSON[3], L. ELLERBY[3], L. GAN[1,2];
[1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]UCSF, San Francisco, CA; [3]Buck Inst., Novato, CA

Abstract: Abnormal accumulation of tau in the brain is linked to severe and debilitating cognitive dysfunction in tauopathies such as Alzheimer's disease (AD). In transgenic mouse models of tauopathy, tau causes memory loss associated with disease; however, the mechanisms by which tau affects neuronal function and promotes cognitive decline are unclear. Using mass spectrometry analysis we detected two lysines on tau, K274 and K281, that were acetylated in human AD brain. We then generated two monoclonal antibodies that selectively recognize these acetylated lysines and investigated the levels of acetylated tau (ac-tau) in AD brains. Human AD cases that were clinically diagnosed with severe dementia had a significantly higher proportion of ac-tau in the brain than non-demented cases. We also found that ac-tau was enhanced in the hippocampus of an AD mouse model expressing human wild-type tau (wt-tau). To determine how ac-tau impacts cognition and the brain we made transgenic mice using the prion promoter to drive neuronal expression of human wt-tau or human tau with mutations to substitute K274 and K281 with glutamines (tauKQ) to mimic the structure and neutral charge of acetylated lysines. In a context discrimination task, we found that mice expressing tauKQ displayed a deficit in pattern separation memory, which is encoded by the dentate gyrus. Electrophysiological recordings from acute hippocampal slices revealed that the expression of long-term potentiation (LTP) in dentate granule cells (DGCs) was impaired in tauKQ mice but not wt-tau mice. We found that tauKQ inhibits the postsynaptic insertion of AMPA-type glutamate receptors (AMPARs) during LTP. We next observed that the polymerization of filamentous actin (F-actin) in DGC spines following LTP induction was impaired in tauKQ mice. Application of jasplakinolide, a drug that stabilizes actin polymerization, during recordings restored LTP in tauKQ mice indicating that tauKQ obstructs activity-dependent AMPAR insertion by disrupting actin cytoskeletal dynamics. Furthermore, we found that the loss of KIBRA, a postsynaptic scaffold protein, underlies the synaptic plasticity deficit triggered by ac-tau. Together, our results suggest that aberrant acetylation of tau on K274 and K281 contributes to memory loss and obstructs synaptic plasticity by inhibiting signaling that regulates postsynaptic actin cytoskeletal dynamics.

Disclosures: **T.E. Tracy:** None. **P. Sohn:** None. **S. Minami:** None. **C. Wang:** None. **Y. Zhou:** None. **D. Le:** None. **Y. Li:** None. **I. Lo:** None. **R. Ponnusamy:** None. **B.W. Gibson:** None. **L. Ellerby:** None. **L. Gan:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.10/C43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH K99 Grant AG044469

BrightFocus Foundation

Title: Dysregulation of eukaryotic elongation factor 1A expression and synaptic plasticity impairments in Alzheimer's disease

Authors: *B. C. BECKELMAN[1], T. MA[2,3,4];
[1]Neurosci. Grad. Program, Wake Forest Sch. of Med., Winston Salem, NC; [2]Intrnl. Med., [3]Dept. of Physiol. and Pharmacol., [4]Dept. of Neurobio. and Anat., Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Alzheimer's disease (AD) is the most common form of dementia in the elderly and is quickly rising to epidemic status. Meanwhile, the molecular mechanisms associated with the disease are still elusive, which hinders our ability to search for therapeutic targets. Previous studies have suggested that eukaryotic elongation factor 1A (eEF1A) upregulation is critical for maintenance of hippocampal long-term potentiation (LTP), a synaptic model for learning and memory. Further, eEF1A synthesis is controlled by the mammalian target of rapamycin complex 1 (mTORC1) pathway, which has been linked to LTP impairments in certain mouse models of AD. Here, we explored the dysregulation of eEF1A in AD pathophysiology using biochemical and electrophysiological methods. First, we observed that basal eEF1A levels are downregulated in hippocampi of APP/PS1 AD model mice and postmortem human AD patients. In contrast, no AD-related change of eEF1A levels was found in cerebellum. Furthermore, eEF1A upregulation in hippocampal slices associated with chemical-LTP induction was blunted in AD model mice. Lastly, brain-specific knock-down of tuberous sclerosis complex 2 (TSC2), a negative regulator of mTORC1, rescued the deleterious effects of amyloid beta (Aβ) on hippocampal LTP. In summary, our studies demonstrated impairments of neuronal plasticity-related eEF1A regulation, thus, revealing a potential novel therapeutic avenue for AD and other aging-related memory deficits.

Disclosures: B.C. Beckelman: None. **T. Ma:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.11/C44

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Modeling of normal and pathological synaptic plasticity related to amyloid beta effects

Authors: *P. ÉRDI[1], T. MATSUZAWA[2], M. GHANNAM[2], L. ZALÁNYI[3];
[2]Ctr. for Complex Systems Studies, [1]Kalamazoo Col., Kalamazoo, MI; [3]Wigner RCP, RMI, Hung. Acad. Sci., Budapest, Hungary

Abstract: As suggested by Palop and Mucke (2010), amyloid beta (Aβ) peptides might have a concentration-dependent dual control effect on excitatory synapses: reduced presynaptic efficacy, presynaptic facilitation and postsynaptic depression appear for low, intermediate and high Aβ concentrations, respectively. Pathologically elevated Aβ impair long term potentiation (LTP) and enhances long term depression (LTD) related to the partial block of N-Methyl-D-aspartate (NMDA) and metabotropic glutamate receptor 5 (mGLur5) receptors. A computational model is developed to explain findings and hypotheses. The model (i) describes the induction and maintenance of long term phenomena by combining a calcium dependent models of bidirectional synaptic plasticity (Shouval 2002} of the induction and a simplified phenomenological model of maintenance motivated by Smolen 2012; (ii) takes into account the effects of Aβ. The calcium control hypothesis assumes that a large calcium transient induces LTP, whereas moderate increase in the calcium concentration induces LTD. The process is controlled by the Ω vs calcium concentration function, which determines both the sign and the magnitude of the change of the synaptic strength. To describe the maintenance mechanism a positive feedback effect is incorporated. Here the synaptic weight is set as a product of two variables with separate dynamics: the first represents the amount of phosphorylated AMPA receptors that are functionally incorporated into postsynaptic sites; the second implements the number of receptors available for incorporation. We couple the two timescales by a phenomenological model of a signaling process: the relatively rapid calcium dynamics induces slow protein expression processes to control the LTP/LTD the maintenance (and expression). Introducing a functional spatial dependence for Ω function we model different levels of Aβ accumulation, which induces impairment of synaptic transmission. This spatial dependence implements that pathologically elevated Aβ may indirectly cause a partial block of NMDARs.Depression of excitatory synapses by high Aβ levels requires activation of mGluR- and NMDAR-dependent LTD pathways. Simulation results describe: a.: Aβ induced synaptic facilitation and occlusion of LTD, b., Aβ induced mGluR and NMDAR dependent LTD facilitation, c: Aβ induced LTP impairment. The next open question to be answered is how elevated Aβ and impaired synaptic plasticity implies destabilization of neural network activity and generation of epileptiform activities.

Disclosures: **P. Érdi:** None. **T. Matsuzawa:** None. **M. Ghannam:** None. **L. Zalányi:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.12/C45

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ministerio de Economia y Competitividad (SAF2014-59697-R)

CIBERNED (CB06/05/0042)

Fundació Marato TV3 (2014- 3610)

Generalitat de Catalunya (SGR2009-1231)

Title: Analysis of synaptic-related miRNAs expression in experimental models of Alzheimer's disease

Authors: A. MIÑANO-MOLINA[1,2], D. SIEDLECKI[1], A. OTXOA DE AMÉZAGA[1], J. CATALA[1], C. SAURA[1,2], *J. RODRIGUEZ-ALVAREZ[1,2];
[1]Inst. De Neurociencies/ UAB, Barcelona, Spain; [2]CIBERNED, Barcelona, Spain

Abstract: MicroRNAs are a group of small non-coding RNAs that regulate gene expression post-transcriptionally. Recent studies have shown that deregulation of specific microRNAs could be involved in the development of Alzheimer's disease (AD). However, few studies exploring the relationship between microRNAs deregulation in AD and synaptic plasticity exist despite the involvement of some microRNAs in synaptic plasticity. Since it is believed that alterations in synaptic function are related to mild cognitive impairment, it is feasible to hypothesize that alterations in plasticity-related microRNAs could underlie AD progression. Here, levels of a small number of microRNAs involved in the regulation of AMPAR function were examined in mice hippocampal cultures, an AD mice model, where we reported previously changes in AMPAR regulation related with early deficits in learning and memory processes, and in human samples. We found increases in miR-181c-5p (~40%), miR-210-3p (>60%) and miR-92a-3p (~25%) expression but not in miR-181a-5p after oAβ treatment in primary hippocampal neurons. Similar changes in miR-181c-5p and miR-92a-3p were confirmed in the entorhinal cortex of APPSw,Ind transgenic mice. However no significant changes were observed in the hippocampus in these mice. Moreover, the analysis of hippocampal human samples at different Braak stages, show an increase in miR-181c-5p and miR-92a-3p levels during AD progression. These findings indicate a possible relationship between miR-181c-5p and miR92a-3p and the reported changes in glutamate receptor levels and early learning and memory deficits in the APPSw,Ind transgenic mice. Our results suggest that those microRNAs involved in synaptic plasticity might be important factors that contribute to AD neuropathology progress.

Disclosures: A. Miñano-Molina: None. **D. Siedlecki:** None. **A. Otxoa de Amézaga:** None. **J. Catala:** None. **C. Saura:** None. **J. Rodriguez-Alvarez:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.13/C46

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG042475

NIH Grant AG027219

NIH Grant AG028383

NIH Grant AG14449

Alz Assoc NIRG-11-198378

Title: Oxidative stress and synaptic protein loss in preclinical Alzheimer's disease

Authors: *S. W. SCHEFF[1], M. A. ANSARI[1], E. J. MUFSON[2];
[1]Ctr. Aging, Univ. Kentucky, Lexington, KY; [2]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Synaptic dysfunction is one of the hallmarks of Alzheimer's disease (AD) and a seminal event leading to amnestic mild cognitive impairment (aMCI). The precise etiology of synaptic failure remains to be identified although there is strong evidence that oxidative stress, which occurs early in the disease process, may play an important role. Detailed neuropathological analysis and quantitative imaging has shown that the medial temporal lobe (hippocampus, entorhinal cortex, transentorhinal cortex) is one of the earliest brain regions affected in the disease progression. These studies suggest a possible protracted preclinical phase of the disease. We have already shown a loss of synapses in the hippocampus in the aMCI phase. The present study was designed to test whether or not synaptic change occurs in possibly a pre-aMCI or preclinical stage of AD. Human short post-mortem hippocampal tissue was obtained from three different cohorts: (1) individuals with low or no AD-type pathology (LP) and no cognitive impairment (NCI), (2) individuals with high AD-type pathology (HP) and NCI, (3) individuals with aMCI. Changes in several different key synaptic proteins were analyzed along with possible changes in markers of oxidative stress. Compared to the age- and postmortem-matched LP-NCI cohort, individuals with HP-NCI and aMCI manifested a significant increase in numerous markers of oxidative stress. The hippocampal analysis also revealed a significant decline in key synaptic proteins. Elevated oxidative stress strongly correlated levels of the different synaptic proteins. As the oxidative stress increased, the level of synaptic proteins decreased. These results support the idea that in the preclinical stage of the disease, there is already a defect in synaptic structure that may be related to levels of oxidative stress. The fact that the HP-NCI group continues to manifest adequate cognitive ability may be evidence for cognitive reserve in this cohort.

Disclosures: S.W. Scheff: None. **M.A. Ansari:** None. **E.J. Mufson:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.14/C47

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ministry of Health and Welfare Grant HI14C2229

Korea Institute of Oriental Medicine Grant K15310

Title: Soluble Aβ oligomers disrupt homeostasis of synaptic vesicle pool among synapses by inhibiting inter-boutonal synaptic vesicle movements

Authors: D. PARK[1], M. NA[2], *S. CHANG[3];
[1]Dept. of Physiol. and Biomed. Sci., [2]Dept. of Physiol. & Biomed. Sci., [3]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Soluble Aβ oligomer (oAβ) has recently been recognized as a major causative factor in the synaptic dysfunction associated with Alzheimer's disease (AD), yet the molecular mechanisms concerning its presynaptic regulation remain poorly understood. Here, using a quantum-dot based individual synaptic vesicle (SV) probe, we found that acute treatment of cultured rat hippocampal neurons with nanomolar concentrations of oAβ dramatically reduced the interboutonal SV trafficking, without affecting mitochondria transport and the integrity of microtubule structures. Neurons derived from AD transgenic mice also showed defected interboutonal trafficking. We further found that oAβ increased presynaptic Ca2+ levels and subsequent phosphorylation of synapsin. STO-609, blocked oAβ-induced synapsin phosphorylation, and neutralized the effect of oAβ. oAβ treatment induced significant heterogeniety of SV pool size among synapses. Our results indicated that oAβ increases presynaptic Ca2+, which resulted in synapsin phosphorylation, releasing SV and actin from synapsin and subsequently inhibiting interboutonal SV trafficking. By disrupting the SV reallocation among synapses, oAβ may disable neurons from adjusting synaptic weights among synapses that could contribute to homeostatic presynaptic plasticity.

Disclosures: **D. Park:** None. **M. Na:** None. **S. Chang:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.15/C48

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ANR

France Alzheimer

Fundação para a Ciência e Tecnologia

Title: Early synaptic deficits in Alzheimer's disease involve neuronal adenosine A2A receptors

Authors: *S. VIANA DA SILVA[1,2], M. G. HABERL[3], P. BETHGE[4], C. LEMOS[5], A. FRICK[3], V. NÄGERL[4], R. A. CUNHA[5], C. MULLE[4];
[1]CNRS IINS UMR 5297, Bordeaux, France; [2]BEB PhD program CNC Coimbra, Univ. of Coimbra, Coimbra, Portugal; [3]Univ. of Bordeaux, Neurocentre Magendie, INSERM U862, Bordeaux, France; [4]Univ. of Bordeaux, Interdisciplinary Inst. for Neuroscience, CNRS UMR 5297, Bordeaux, France; [5]CNC-Center for Neurosci. and Cell Biology, Univ. of Coimbra, Coimbra, Portugal

Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized clinically by progressive memory loss eventually resulting in dementia. Over the course of the AD amyloid-beta (AB) deposition forms insoluble amyloid plaques but synapse loss is known be better correlated with the progression of the disease. Although the exact role of AB is not fully understood, recent evidence suggests that subtle alterations of synaptic transmission precede neuronal degeneration in the AD. Early disturbance of synaptic processes involved in learning and memory have been reported in several transgenic mouse models. Synaptic plasticity in the autoassociative network of recurrent connections among hippocampal CA3 pyramidal cells is thought to enable episodic memories storage. At the early onset of cognitive deficits in a mouse model of Alzheimer's disease (AD), associative long-term synaptic potentiation (LTP) is abolished in CA3 pyramidal cells. This is caused by activation of up-regulated neuronal adenosine A2A receptors (A2AR) rather than by dysregulation of NMDAR signaling or structural synaptic modifications. Neutralization of A2AR by acute pharmacological inhibition, or downregulation driven by shRNA interference in a single postsynaptic neuron restore associative CA3 LTP. Accordingly, treatment with A2AR antagonists reverts short-term memory deficits.

Disclosures: S. Viana Da Silva: None. **M.G. Haberl:** None. **P. Bethge:** None. **C. Lemos:** None. **A. Frick:** None. **V. Nägerl:** None. **R.A. Cunha:** None. **C. Mulle:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.16/C49

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Santa Casa da Misericordia

QREN

Title: Adenosine A2A receptor blockade reverts installed deficits of hippocampal-dependent memory and synaptic plasticity in animal models of Alzheimer's disease

Authors: *R. A. CUNHA[1], J. P. LOPES[2], C. LEMOS[2], A. M. CARVALHO-DA-SILVA[2], A. PLIASSOVA[2], F. Q. GONÇALVES[2], N. J. MACHADO[2], P. M. CANAS[2], P. AGOSTINHO[2]; [1]CNC -Center For Neurosci. and Cell Biol., Coimbra, Portugal; [2]CNC-Center for Neurosci. and Cell Biol., Coimbra, Portugal

Abstract: Chronic consumption of caffeine prevents age-related memory dysfunction and is inversely correlated with the incidence of Alzheimer's disease (AD) and animal models confirmed this prophylactic benefit of caffeine (J Alzheimers Dis 20:S95). In accordance with the evidence that caffeine mostly acts through the antagonism of adenosine receptors at non-toxic doses, animal studies allowed defining that selective antagonists of adenosine A2A receptors (A2AR) mimic these neuroprotective effects of caffeine (J Alzheimers Dis 20:S95). The increased density of A2AR upon memory dysfunction, together with the ability of A2AR agonists or activation of A2AR transduction systems to trigger memory impairment and disrupt hippocampal long-term potentiation (LTP, the purported neurophysiological basis of memory) further suggest a causal role for A2AR over-functioning as a trigger of memory impairment. However, it has not been established if A2AR blockade can also revert pre-installed memory deficits. This was first tested in adult mice with memory impairment (object displacement, modified Y-maze), 15 days after exposure to β-amyloid peptides (Aβ1-42, 2 nmol, icv), where A2AR were up-regulated in particular in glutamatergic terminals that undergo selective degeneration (Neuropharmacol 76:51). Slices from these Aβ-treated mice display a reduced LTP in CA1 synapses. Notably, a 30 min superfusion with the A2AR antagonist, SCH58261 (50 nM), reverted LTP amplitude deficits to values similar to sham-operated mice without memory impairments. In another AD animal model (3xTg mice), memory impairment was observed at 4.5 months, which was accompanied by a lower LTP amplitude in the CA1 area together with a decreased density of synaptic markers in the hippocampus (synaptophysin, SNAP25 and vGluT1, but not vGAT). These 3xTg mice also displayed an increased density of A2AR in hippocampal synaptic membranes. Notably a 3-weeks treatment with SCH58261 (0.1 mg/kg ip, daily) reverted the deficits of memory performance of 3xTg mice, without altering the 'normal' memory performance of WT mice. Furthermore, slices from SCH58261-treated mice displayed an LTP amplitude similar to that found in WT mice and larger than that found in vehicle-treated 3x-Tg mice. Likewise, the decrease of synaptic markers in the hippocampus of vehicle-treated 3xTg mice was not present in SCH58261-treated 3xTg mice. These results further re-enforce the key role of A2AR in the emergence of the early features of AD and prompt the suggestion that A2AR antagonists might not only be a prophylactic but also a promising therapeutic strategy to manage early AD.

Disclosures: **R.A. Cunha:** None. **J.P. Lopes:** None. **C. Lemos:** None. **A.M. Carvalho-da-Silva:** None. **A. Pliassova:** None. **F.Q. Gonçalves:** None. **N.J. Machado:** None. **P.M. Canas:** None. **P. Agostinho:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.17/C50

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

NARSAD Young Investigator Award, Brain & Behaviour Research Foundation

Title: Sigma-1 receptor involvement in an animal model of Alzheimer’s disease

Authors: ***M. A. SNYDER**, K. MCCANN, M. M. J. LALANDE, R. BERGERON; Ottawa Hosp. Res. Inst., Ottawa, ON, Canada

Abstract: Background: Alzheimer’s disease (AD) is characterized by the progressive loss of neurons in the cortex and hippocampus, leading to cognitive decline. While a small percentage of AD is hereditary, the vast majority of cases are sporadic, composed of both genetic and non-genetic risk factors. Despite decades of research, mostly focused on the amyloid beta (Abeta) synthesis pathway, there is still no cure and current therapies fail to significantly alter the disease course. Thus, new targets for therapeutics are desperately needed. One intriguing area of investigation has focused on the sigma 1 receptor (Sig1R). Sig1Rs are proposed to play a role in the pathogenesis of AD for a number of reasons: 1) genetic polymorphisms of the Sig1R confer risk for AD; 2) Sig1R expression is reduced in AD; 3) Sig1R agonists are neuroprotective and anti-amnesic in AD mouse models; and finally 4) Aricept, the most prescribed drug for AD patients, has nanomolar affinity for Sig1R. Together these results led us to question whether loss of Sig1R function is involved in the underlying etiology of AD. **Rationale and Hypothesis:** The role of Sig1Rs in regulating calcium homeostasis under cellular stress is particularly interesting given the importance of calcium homeostasis for proper cell functioning. Moreover, alterations in calcium homeostasis are observed during AD and normal aging. We hypothesize that loss of Sig1R will exacerbate the cognitive and synaptic deficits in AD by potentiating perturbations in calcium homeostasis. To verify our hypothesis we used an *in vivo* knockout (KO) mouse model, the Sig1R KO mice. **Preliminary data:** We first explored Sig1R’s ability to regulate calcium using whole-cell electrophysiology and examining the post-burst afterhyperpolarization (AHP). This paradigm is often used as a proxy for changes in calcium homeostasis. We found that the Sig1R agonist (+) pentazocine increases the AHP. We next explored how loss of Sig1R affects calcium regulation, physiology, and behavior in an AD mouse model. We used the $Abeta_{25-35}$

infusion model as it recapitulates the process of sporadic AD, including impairments in learning, synaptic plasticity, Abeta plaque deposits, and cell loss in the hippocampus. Wild-type (WT) and Sig1R KO mice were infused intracerebroventricularly with $Abeta_{25-35}$. We found that $Abeta_{25-35}$ impairs performance on spontaneous alternation in the Y maze, reduces the magnitude of long-term potentiation, reduces neuronal excitability, and increases the AHP in Sig1R KO compared to WT. Future experiments will determine how calcium homeostasis is altered and whether reversing changes to calcium will prevent changes to physiology and behavior.

Disclosures: **M.A. Snyder:** None. **K. McCann:** None. **M.M.J. Lalande:** None. **R. Bergeron:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.18/C51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MRC Grant MR/J011851/1

ARUK Grant SRF2013-7

Title: Effect of the pentraxins on neurons and microglia in the CNS

Authors: *T. BENWAY, D. M. CUMMINGS, E. FOSTER, W. LIU, M. V. YASVOINA, F. A. EDWARDS, D. A. SALIH;
Univ. Col. London, London, United Kingdom

Abstract: The pentraxins are neuromodulators that are linked to Alzheimer's disease (AD) (for review, Osera et al. Ageing Res. Rev. 11:189, 2012). In particular, neuronal pentraxin 1 (NPTX1) mediates neurite degeneration induced by amyloid-β when applied to neurons in culture (Abad et al. J.Neurosci. 26:12735, 2006) and NPTX2 has been shown to regulate inflammation, a key process that is dysregulated in AD (Miskimon et al. J.Neuroimmunol. 274:86, 2014). At the synapse, NPTX1, NPTX2 and the NPTX receptor (NPTXR) interact to play a key role in plasticity by clustering AMPA receptors (O'Brien et al., J.Neurosci. 22:4487, 2002). We have studied effects of neuronal pentraxins using patch clamp recording of wild type mouse hippocampal CA1 neurons in organotypic slices. Application of nanomolar concentrations of any one of the human neuronal pentraxins over 7 days reduced paired-pulse ratios of Schaffer collateral synapses, in a dose-dependent manner. This indicates an increase in glutamate release probability, with different potencies of the three pentraxins: NPTX1>NPTXR>NPTX2. Interestingly, the increased probability of release is similar to that observed in transgenic mice expressing human genes that cause familial AD (Cummings et al., Brain in press July, 2015). In agreement with Abad et al. (2006) our preliminary results confirm

that NPTX1 overexpression inhibits the capability of neurons to form complex dendritic processes *in vitro*. Interestingly our recently published database www.mouseac.org indicates changes in gene expression of the neuronal pentraxins at different ages in two different dementia-related mouse models (human APPswe+PSEN1M146V or TauP301L, Matarin et al., Cell Rep 10:633-644 (2015). In relation to the role of inflammation in AD, we have shown that the NPTXR is expressed in the BV2 microglia cell line. Current research aims further to characterize the involvement of neuronal pentraxins in the immune response by using organotypic hippocampal cultures, where a greater extent of brain physiology can be examined. Also, given that NPTX1 forms complexes at the synapse with both NPTX2 and NPTXR, we are using overexpression studies to gain more information about the interactions of these neuronal pentraxins with each other and with peripheral pentraxins that have also been suggested to have a role in AD (see Osera et al., 2012 for review). All work with animals carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

Disclosures: **T. Benway:** None. **D.M. Cummings:** None. **E. Foster:** None. **W. Liu:** None. **M.V. Yasvoina:** None. **F.A. Edwards:** None. **D.A. Salih:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.19/C52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Weston Foundation

Title: Rapid modulation of protein expression in the rat hippocampus following deep brain stimulation of the fornix

Authors: ***E. GONDARD**[1], H. N. CHAU[1], A. MANN[1], T. S. TIERNEY[2], C. HAMANI[1,3], S. K. KALIA[1], A. M. LOZANO[1];
[1]Neurosurg., Toronto Western Hosp. - Univ. of Toronto, Toronto, ON, Canada; [2]Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA; [3]Res. Imaging Centre, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Background: The forniceal area is currently being evaluated as a target for deep brain stimulation (DBS) to improve cognitive function in patients with Alzheimer’s disease. The molecular changes at downstream targets within the stimulated circuit are unknown. Objective: To analyze the modulation of hippocampal protein expression following one hour of fornix DBS in the rat. Methods: Animals underwent bilateral forniceal DBS for one hour and sacrificed at different time-points after the initiation of the stimulation (1h, 2.5h, 5h, 25h). Bilateral hippocampi were isolated for western blot analyses. Results: Forniceal DBS led to a dramatic

elevation of cFos post-stimulation, suggesting that forniceal DBS activates the hippocampus. There was also a significant increase in candidate proteins including several trophic factors, such as brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) but not glial cell-derived neurotrophic factor (GDNF). There was in addition, increased expression of the synaptic markers growth associated protein 43 (GAP-43), synaptophysin and α-synuclein. No changes were observed at the studied time-points in Alzheimer's-related proteins including amyloid precursor protein (APP), tau, phosphorylated tau (ptau), or selected chaperone proteins (HSP40, HSP70 and CHIP). Conclusions: Forniceal DBS triggers hippocampal activity and rapidly modulate the expression of neurotrophic factors and markers of synaptic plasticity known to play key roles in memory processing. The clinical effects of DBS of the fornix may, in part, be mediated by producing changes in the expression of these proteins.

Disclosures: E. Gondard: None. **H.N. Chau:** None. **A. Mann:** None. **T.S. Tierney:** None. **C. Hamani:** None. **S.K. Kalia:** None. **A.M. Lozano:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.20/C53

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Increased hippocampal extracellular matrix causes cognitive decline in a mouse model of Alzheimer's disease

Authors: *C. HELDRING[1], M. J. VEGH[1], I. PALIUKHOVICH[1], M. J. M. SASSEN[1], K. LI[1], P. VAN NIEROP[1], E. M. HOL[2], A. B. SMIT[1], R. E. VAN KESTEREN[1];
[1]Ctr. for Neurogenomics and Cognitive Res., VU Univ. Amsterdam, Amsterdam, Netherlands; [2]Dept. of Translational Neuroscience, Brain Ctr. Rudolf Magnus, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease and nowadays the most common cause of dementia. AD is anatomically characterized on the macroscopic level by brain atrophy and on the microscopic level by amyloid plaques due to increased beta amyloid levels in the brain. The earliest clinical signs of the disease however are memory loss and cognitive impairment. These functional alterations are thought to precede the anatomical hallmarks of the disease and are currently not well understood in molecular and cell physiological terms. In this study we used the $APP_{swe}/PS1_{dE9}$ (APP-PS1) transgenic mice to study the potential causes of initial memory loss and cognitive decline in AD. We found that 3 months old APP-PS1 mice lack amyloid plaques in the brain, but do show an impairment in hippocampus-dependent spatial memory, which is accompanied by impaired hippocampal LTP induction and an upregulation of synaptic extracellular matrix (ECM) proteins. Intra-hippocampal infusion of the ECM degrading

enzyme chondroitinase ABC (chABC) rescued both memory and LTP impairments. To understand the mechanisms behind this chABC-mediated rescue of synaptic plasticity we are currently analysing the molecular composition of the hippocampal ECM of APP-PS1 and wildtype mice. We found that chABC treatment of the hippocampal slices releases potential novel ECM-associated proteins. Comparative analysis in APP-PS1 and wildtype mice of chABC-induced protein release *in vitro* and chABC-induced effects on synaptic ECM composition *in vivo* may result in the discovery of potential new drug targets for treatment of early cognitive impairments in AD.

Disclosures: C. Heldring: None. **M.J. Vegh:** None. **I. Paliukhovich:** None. **M.J.M. Sassen:** None. **K. Li:** None. **P. van Nierop:** None. **E.M. Hol:** None. **A.B. Smit:** None. **R.E. van Kesteren:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.21/C54

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG014449

Title: Precuneus dendritic spine reduction is associated with cognitive impairment in MCI and early Alzheimer’s disease

Authors: *Z. MI[1,4], E. E. ABRAHAMSON[1,4], A. Y. RYU[1], L. SHAO[1], J. K. KOFLER[2], K. N. FISH[3], R. A. SWEET[1,3,5], E. J. MUFSON[6], M. D. IKONOMOVIC[1,3,4];
[1]Dept. of Neurol., [2]Pathology, [3]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [4]Geriatric Res. Educ. and Clin. Ctr., [5]VISN 4 Mental Illness Research, Educ. and Clin. Ctr., VA Pittsburgh Healthcare Syst., Pittsburgh, PA; [6]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Precuneus, a component of the neocortical default mode network, contributes to episodic memory function which is disrupted early in Alzheimer disease (AD). However, the relationship between precuneus neuropathology, including synaptic alterations, and cognitive decline in AD is unclear. A recent electron microscopy study reported that numbers of total synapses in precuneus lamina III were stable in mild cognitive impairment (MCI) and reduced in AD, but were not correlated with cognitive performance (Scheff, 2013). Here, we applied unbiased stereologic principles with quantitative confocal microscopy to analyze spinophilin immunofluorescent dendritic spines in lamina III of precuneus from 27 Rush Religious Order Study participants who died with clinical diagnosis of no cognitive impairment (NCI, MMSE 26-30), MCI (MMSE 22-30) or early AD (MMSE 17-28). Density and immunofluorescence intensity of spinophilin-positive dendritic spines were compared among the three clinical groups

and correlated with cognitive measures (MMSE, global cognitive and episodic memory tests) and precuneus neuropathology burden, assessed as percent area covered with 6-CN-PiB positive plaques or PHF1-immunoreactive p-Tau pathology. We observed that in the precuneus from AD cases, immunofluorescence intensity of spinophilin-positive dendritic spines was lower compared to the MCI (but not the NCI) group, while a reduction in spine densities did not reach statistical significance. Across all cases in the study, lower values of spinophilin-positive dendritic spine measures correlated significantly with lower MMSE scores. Lower spinophilin immunofluorescence intensity also correlated significantly with poorer global cognitive and episodic memory test scores. Precuneus 6-CN-PiB positive plaque burden was greater in AD than in the NCI (but not the MCI) group, and across all cases it correlated significantly with global cognitive and episodic memory scores. Precuneus PHF1 pathology burden was not different among the clinical groups and did not correlate with cognitive measures. Measures of spinophilin-positive dendritic spines in the precuneus did not correlate with 6-CN-PiB positive plaques or tau positive pathology burden. Our results demonstrate that cognitive decline in AD is associated with reduced dendritic spines and increased PiB binding in the precuneus, but the two measures did not appear to be related. Perhaps, the observed post-synaptic alterations are due to non-fibrilized (oligomeric) forms of Aβ and/or tau, invisible to currently available PET radiotracers for detecting amyloid pathology *in vivo*.

Disclosures: **Z. Mi:** None. **E.E. Abrahamson:** None. **A.Y. Ryu:** None. **L. Shao:** None. **J.K. Kofler:** None. **K.N. Fish:** None. **R.A. Sweet:** None. **E.J. Mufson:** None. **M.D. Ikonomovic:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.22/C55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Weston Foundation

Title: Chronic deep brain stimulation enhances learning in young mice

Authors: ***A. MANN**[1], C. HAMANI[1,2], A. M. LOZANO[1];
[1]Neurosurg., Toronto Western Hospital-UHN, Toronto, ON, Canada; [2]Res. Imaging Centre, Ctr. for Addiction and Mental Health,, Toronto, ON, Canada

Abstract: Deep brain stimulation (DBS) is a well-established method of focally and titratably modulating neural activity in patients with several disease processes, including movement disorders, psychiatric illness, and pain. Despite its widespread and successful use in Parkinson's disease, and promise in Alzheimer's disease, much of the mechanisms by which DBS exerts its

effects remain unknown. We have previously shown that acute DBS can modulate hippocampal inputs and increase neurogenesis in rodent models. To partially mimic and better model DBS conducted in clinical settings, we assessed the impact of chronic DBS in naïve young mice. We used 2-month old C57bl/6 mice and bilaterally implanted electrodes in the entorhinal cortex (EC). Mice were stimulated for 7 hours per day with 50 μAmp using a frequency of 130 Hz and pulse width of 90 μSec, for 25 days. After 25 days of stimulation mice were assessed for changes locomotor activity in the Open Field and learning and memory using the Morris water maze (MWM). We found that chronically stimulated mice were significantly faster at learning the MWM task when compared to Control mice. However, no significant difference was seen during the probe test. In addition, no differences were observed in the Open Field. This is the first study to show that 25 days of EC stimulation improves learning behaviour through direct activation of the hippocampus.

Disclosures: A. Mann: None. **C. Hamani:** None. **A.M. Lozano:** None.

Poster

300. Synaptic Pathology in Alzheimer's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 300.23/C56

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DA 09082, DA020129, Philadelphia Health Education Corporation

Title: Amyloid Beta co-localizes with dopamine beta hydroxylase in dense core vesicles of cortical noradrenergic terminals: anatomical evidence for putative regulated co-secretion

Authors: *J. ROSS[1], B. A. S. REYES[1], A. SAUNDERS[2], E. J. VAN BOCKSTAELE[1]; [1]Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Biol., Drexel Univ. Col. of Arts and Sci., Philadelphia, PA

Abstract: Over 5 million Americans are currently suffering from Alzheimer's disease (AD), the most common cause of dementia. Currently, AD has no effective treatment and represents a substantial multi-billion dollar cost burden to the health care system. AD is a neurodegenerative disorder classically characterized by histological features including amyloid beta (Aβ) peptide aggregates known as senile plaques, and hyper-phosphorylated tau protein aggregates known as neurofibrillary tangles (NFT). One of the earliest regions to become dysregulated in AD is the locus coeruleus (LC), the dorsal pontine nucleus that provides the neurotransmitter norepinephrine (NE) to almost all levels of the neuraxis. The dysregulation of the LC-NE system is thought to contribute to multiple aspects of AD pathogenesis. For example, it has been shown that degeneration of the LC and subsequent decreases in NE create a permissive environment for Aβ-induced inflammation. While the detrimental effects of LC degeneration have been the

subject of intense investigation, there are still extensive gaps in our knowledge regarding the interaction of NE in terminal regions of the LC and Aβ at the synapse. *In vitro* studies in neuron-like chromaffin cells have localized Aβ, BACE, and gamma secretases to large dense core vesicles (LDCVs) (Toneff et al., 2013, Peptides, 46:126-35) and further, have demonstrated regulated co-secretion of Aβ with catecholamine neurotransmitters also present within these vesicles. In the present study, we examined the cellular substrates for interactions between Aβ and dopamine beta hydroxylase (DBH), a marker of noradrenergic axon terminals in the rat frontal cortex (FC) using light, immunofluorescence and electron microscopy. Three antibodies for Aβ have been used to evaluate the distribution of various isoforms of the peptide within noradrenergic terminals. Our preliminary immunofluorescence data show co-localization of Aβ and DβH with similar distribution between antibodies, further validating the presence of Aβ in noradrenergic terminals. Using high resolution immunoelectron microscopy, semi-quantitative preliminary analysis revealed that 19% of DBH-containing axon terminals (54/281) also exhibited Aβ-immunogold silver particles, of which approximately 68% are associated with dense core vesicles (37/54). Aβ is also localized to approximately 13% of somatodendritic processes (42/323), of which approximately 14% (6/42) are post-synaptic to NE-containing axon terminals. An on-going study aims to further characterize the presence of Aβ in the LDCVs of noradrenergic projections using chromogranin A, a marker for the LDCV.

Disclosures: J. Ross: None. **B.A.S. Reyes:** None. **A. Saunders:** None. **E.J. Van Bockstaele:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.01/C57

Topic: C.03. Parkinson’s Disease

Support: COEN (Centres of Excellence in Neurodegeneration) grant

Italian Ministry of Health (Ricerca Corrente)

Title: Defective glucocerebrosidase in GBA mutant Parkinson's disease fibroblasts is rescued by chemical chaperone ambroxol through modulation of lysosomal factors

Authors: *F. BLANDINI, C. GHEZZI, R. ZANGAGLIA, G. LEVANDIS, C. PACCHETTI, G. AMBROSI;
Ctr. for Res. in Neurodegenerative Dis., Natl. Neurolog. Inst. C.Mondino, Pavia, Italy

Abstract: Introduction: Heterozygous mutations in GBA gene, encoding for lysosomal enzyme glucocerebrosidase (GCase), are a major risk factor for sporadic Parkinson's disease (PD) [1]. Defective GCase has been recently reported in fibroblasts of GBA-mutant PD patients and

pharmacological chaperone ambroxol has been shown to correct such defect [2]. A number of endogenous elements support GCase activity, especially transporter and lysosomal receptor LIMP2 and activator saposin (Sap) C, which have been suggested as possible disease modifiers in PD [3]. Objective: our aim was to further investigate GCase activity, associated lysosomal and proteasomal factors at baseline and after ambroxol administration in fibroblasts from sporadic PD patients, with or without heterozygous GBA mutations, and healthy subjects. Methods: We assessed protein levels of GCase, LIMP2, Sap C and parkin - a central element in PD and cellular proteostasis - by western blotting. We measured activities of GCase and cathepsin D, responsible for Sap C cleavage from precursor prosaposin, using ELISA assays. All analyses were carried out in basal conditions and following exposure to ambroxol. Results: GCase activity was reduced in fibroblasts from GBA-mutant patients and ambroxol corrected this defect, thereby confirming previous results. Ambroxol increased cathepsin D activity, GCase and Sap C protein levels in all groups and LIMP2 protein levels in GBA-mutant PD fibroblasts. Parkin levels were slightly increased only in the PD group without GBA mutations and were not significantly modified by ambroxol. Conclusion: Our study confirms that GCase activity is deficient in GBA-mutant PD patients, further indicating that fibroblasts are a good model to investigate proteolytic dysfunction. Moreover, our study shows that ambroxol-induced rescue of GCase activity is associated with enhanced expression of LIMP2 and Sap C; ambroxol does not interfere with parkin, confirming that this chemical chaperone selectively modulates lysosomal pathways that may be targeted for the development of innovative therapeutic strategies.

Disclosures: F. Blandini: None. **C. Ghezzi:** None. **R. Zangaglia:** None. **G. Levandis:** None. **C. Pacchetti:** None. **G. Ambrosi:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.02/C58

Topic: C.03. Parkinson’s Disease

Support: Spanish Ministry of Economy and Competitiveness-FEDER; grant number: BFU2010-15729

Spanish Ministry of Economy and Competitiveness-FEDER; grant number SAF2014-52300-R

Autonomous Government of Castilla-La Mancha-FEDER; grant: PEIC-2014-006-P

We are indebted to the IDIBAPS, BTCIEN and BIOBANC-MUR Biobanks for the sample and data procurement

Title: Differential neuronal vulnerability by α-synucleinopathy in the human anterior olfactory nucleus in Parkinson's disease

Authors: *I. UBEDA-BANON, A. FLORES-CUADRADO, D. SAIZ-SANCHEZ, C. DE LA ROSA-PRIETO, A. MARTINEZ-MARCOS;
Fac. Med. Ciudad Real-Crib Univ. Castilla-La Mancha, Ciudad Real, Spain

Abstract: Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders, mainly idiopathic and with palliative treatment. Neuropathologically, it is a proteinopathy with aggregates of ubiquitin and α-synuclein called Lewy bodies and neurites. These deposits appear in a predictable sequence of six stages beginning in olfactory structures, particularly in the anterior olfactory nucleus. Recently, it has identified a preclinical (prodromal) period that would begin even decades before symptoms lead to neurological diagnosis, which opens a promising therapeutic window. This period would be characterized by early symptoms such as olfactory deficits that could correlated with neuropathological staging. Further, neurophatological spreading could occur in a prion-like manner through axons and transynaptically. The aim of this study has been, therefore, study the differential vulnerability of neuronal populations induced by α-synuclein in the different divisions of the anterior olfactory nucleus (retrobulbar, cortical anterior medial, cortical anterior lateral, cortical posterior medial and cortical posterior lateral subdivisions). Human neurological tissue from Parkinson's patients (stages 4-5) and healthy age-matched controls were used. Samples and data from patients included in this study, who gave written informed consent, were collected, processed and provided by the IDIBAPS, BTCIEN and BIOBANC-MUR integrated in the Spanish Biobanks Network and were processed following standard operating procedures with appropriate approval of the Ethical and Scientific Committees. Neuronal markers have been used to determine cell loss. Triple immunofluorescence of calretinin, parvalbumin and α-synuclein or calbindin, somatostatin and α-synuclein plus DAPI were carried out to analyze the differential vulnerability of interneuron populations. Neuronal confocal images were used to quantify potential cell loss and co-localization using Zen software from Zeiss. Our results show differences in vulnerability and co-localization of different cell types in the various divisions of the human anterior olfactory nucleus in PD. These results could help to understand neural substrates of non-motor symptoms such as anosmia and how this proteinopathy could propagate through the olfactory system.

Disclosures: I. Ubeda-Banon: None. **A. Flores-Cuadrado:** None. **D. Saiz-Sanchez:** None. **C. De la Rosa-Prieto:** None. **A. Martinez-Marcos:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.03/C59

Topic: C.03. Parkinson's Disease

Support: Spanish Ministry of Economy and Competitiveness-FEDER; grant number: BFU2010-15729

Spanish Ministry of Economy and Competitiveness-FEDER; grant number: SAF2014-52300-R

Autonomous Government of Castilla-La Mancha-FEDER; grant: PEIC-2014-006-P.

We are indebted to the IDIBAPS, BTCIEN and BIOBANC-MUR Biobanks for the sample and data procurement.

This work constitutes part of the Doctoral Thesis of Alicia Flores-Cuadrado.

Title: α-synucleinopathy and differential vulnerability of interneurons in the human amygdala and hippocampus

Authors: A. FLORES-CUADRADO, I. UBEDA-BANON, D. SAIZ-SANCHEZ, C. DE LA ROSA-PRIETO, *A. MARTINEZ-MARCOS;
Fac. Med. Ciudad Real, CRIB Univ. Castilla-La Mancha, Ciudad Real, Spain

Abstract: Olfactory dysfunction, anhedonia and dementia are non-motor symptoms involved in Parkinson's disease (PD). These symptoms might be correlated to the appearance of Lewy bodies and neurites (ubiquitin and alpha-synuclein aggregates) in particular brain areas according to six stages of Braak's neuropathological description. The olfactory bulb, the anterior olfactory nucleus and the dorsal motor nucleus of the vagus nerve are involved at stage 1. However, the amygdala and the substantia nigra are involved at stage 3. Previous to cortical spreading, Lewy bodies appear in the hippocampus at stage 4. It has been described that oxidative stress and calcium-induced excitotoxicity are involved in PD cell death. Apart from pyramidal projection neurons, a number of interneuron subpopulations have been described in the amygdaloid complex and hippocampus. These subpopulations have been characterized based on their expression of calcium-binding proteins such as calretinin, calbindin and parvalbumin and neuropeptides such as somatostatin. The goal of this work has been to analyze the α-synucleinopathy and the differential vulnerability of interneuron populations in the amygdala (cortical, central and basolateral nuclei) and hippocampus (CA3, CA2, CA1 and dentate gyrus). In addition, we evaluated if there are differences between rostral and caudal levels and stages in these particular brain areas. We have analyzed 5 PD post-mortem samples at different stages: 2 (stage 3), 1 (stage 4), 2 (stage 5). Samples and data from patients included in this study, who gave written informed consent, were collected, processed and provided by the IDIBAPS, BTCIEN and BIOBANC-MUR integrated in the Spanish Biobanks Network and were processed following standard operating procedures with appropriate approval of the Ethical and Scientific Committees. α-synuclein and interneuron markers positive-cells were quantified using immunofluorescence procedures. Three photographs per area and multiple Z-stacks were taken in confocal microscope. Binarization and threshold were applied in all pictures using ImageJ software. Co-localization between alpha-synuclein and interneurons were carried out using Zen Software Ortho tool. We observed that Lewy bodies were abundant at stage 3 whereas Lewy

neurites appeared at stage 4 and 5 in CA2. Lewy neurites in basomedial amygdaloid nuclei were higher than other amygdaloid nucleus. This analysis could be helpful to understand given non-motor symptoms of PD as well as mechanism of α-synucleinopathy spreading along the temporal lobe.

Disclosures: A. Flores-Cuadrado: None. **I. Ubeda-Banon:** None. **D. Saiz-Sanchez:** None. **C. De la Rosa-Prieto:** None. **A. Martinez-Marcos:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.04/C60

Topic: C.03. Parkinson's Disease

Title: Activity-dependent neuroprotective protein expression in nigral dopaminergic neurons in sporadic Parkinson's disease

Authors: *Y. CHU, Y. HE, J. H. KORDOWER;
Rush Univ. Med. Ctr., Chicago, IL

Abstract: Activity-dependent neuroprotective protein (ADNP), a vasoactive intestinal peptide (VIP) regulated gene, is essential for brain formation and function. Mutations in ADNP gene are associated with neurodegenerative diseases. It is possible that intracellular alpha-synuclein inclusions, occurring in the context of neurodegenerative diseases, may be due to, or may cause, down-regulation of ADNP expression. The present study investigated alterations of ADNP in dopaminergic neurons in patients with sporadic Parkinson's disease (n =10) and age-matched controls (n=9). Co-localization analyses revealed that ADNP immunoreactivity was severely reduced in both neuromelanin (NM)-laden neurons with or without alpha-synuclein aggregations in PD. Quantitative immunofluorescence intensity measurements in NM-laden nigral neurons further demonstrated that the optical density of ADNP levels was significantly decreased in neurons with (370.79±71.44; $P < 0.01$) and without (388.51±77.30; $P < 0.01$) alpha-synuclein inclusions in Parkinson's disease relative to the age-matched controls (1216.92±51.58). There was no difference between NM-laden nigral neurons with present or absent alpha-synuclein inclusions ($P > 0.05$). Evaluation of ADNP levels with the symptoms of Parkinson's disease progress (H&Y scale) revealed that there were no differences ($P> 0.05$) among H&Y stages II-V, suggesting that reduction of ADNP expression is an early alteration during Parkinson's disease progress. Correlation analyses demonstrated that the decline in ADNP levels was linear with reduction of kinesin heavy chain levels ($r = 0.66$; $P < 0.01$) and dynein light chain tctex type 3 ($r = 0.85$; $P <0.001$). These preliminary data demonstrated that decreased ADNP is independent of alpha-synuclein inclusions, correlated alteration of axonal transport motor protein, and an early event in the pathogenesis of Parkinson's disease.

Disclosures: **Y. Chu:** None. **Y. He:** None. **J.H. Kordower:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.05/C61

Topic: C.03. Parkinson's Disease

Title: Using eye movements to identify early biomarkers of disease progression in Parkinson's patients with and without LRRK2 gene mutations

Authors: ***J. MORRIS**[1], D. C. BRIEN[1], B. C. COE[1], N. VISANJI[2], T. GHATE[2], A. E. LANG[2], C. MARRAS[2], D. P. MUNOZ[1];
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Morton and Gloria Shulman Movement Disorders Clin. and the Edmond J Safra Program in Parkinson Dise, Univ. Hlth. Network, Toronto, ON, Canada

Abstract: In some patients with Parkinson's disease (PD), variations of the Leucine-rich repeat kinase 2 (LRRK2) gene have been associated with the development of the disease. Patients with PD exhibit specific deficits when performing anti-saccades, an inhibitory task that requires looking in the mirror location of a peripheral visual stimulus. These deficits include longer reaction times, increased direction errors (erroneously looking at the stimulus), and hypometric saccades. We conducted an interleaved pro- and anti-saccade task with age-matched controls, idiopathic PD patients, and LRRK2 mutation carriers either before (non-manifesting) or after they manifest PD (manifesting carriers). The pro-saccade task (look at the peripheral stimulus) assesses the basic sensory-motor processing of eye movements via automatic tendencies to look at salient visual stimuli, whereas the anti-saccade task assesses the inhibition of this automatic response and generation of a voluntary command to look in the mirror location. Interestingly, preliminary analysis revealed that carriers of pathogenic LRRK2 genetic mutation, who have not yet developed Parkinsonism, had anti-saccade deficits similar to PD patients. More specifically, non-manifesting carriers had longer reaction times during the anti-saccade task, resembling the PD patient group. Unsurprisingly, manifesting carriers performed similarly to patients with Parkinson's without the LRRK2 gene mutation in both reaction times and error measures. Further analysis is essential to identify more pre-symptomatic behavioural biomarkers of PD that accurately predict disease and thus lead to earlier PD detection.

Disclosures: **J. Morris:** None. **D.C. Brien:** None. **B.C. Coe:** None. **N. Visanji:** None. **T. Ghate:** None. **A.E. Lang:** None. **C. Marras:** None. **D.P. Munoz:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.06/C62

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

Title: Parkinson's and Crohn's disease-associated LRRK2 mutations and LRRK2 inhibitors alter type II interferon responses of human peripheral blood monocyte *ex vivo*

Authors: *T. IKEZU[1], H. ASAI[2], S. IKEZU[2], B. WOLOZIN[2], F. FARRAYE[2], Z. WSZOLEK[3]; [1]Pharmacol. and Neurol., [2]Boston Univ. Sch. of Med., Boston, MA; [3]Mayo Clin., Jacksonville, FL

Abstract: The Leucine Rich Repeat Kinase 2 (LRRK2) is a causative gene of familial Parkinson's disease (PD). In addition, the M2397 allele of M2397T polymorphism in LRRK2 gene is genetically associated with sporadic Crohn's disease. LRRK2 is highly expressed in neurons in the central nervous system and in human peripheral blood mononuclear cells (PBMCs), especially CD14+ monocytes. Recent studies suggest that LRRK2 transcription is potently induced by type II interferon (IFN-γ and suppresses the activity of the transcription factor Nuclear Factor of Activated T cells (NFAT) in human immune cells. The putative role of LRRK2 in immune function raises the possibility that LRRK2 contributes to PD by altering brain inflammatory response. We hypothesize that IFN-γ induces LRRK2 gene expression, which inhibits NFAT activity, leading to a resolution of IFN-γ-mediated inflammation, and this may be altered in human monocytes derived from PD or CD patients by LRRK2 mutations. A total of 50 CD and 50 control cases and 15 PD with LRRK2 mutation cases and 20 PD without LRRK2 mutation cases are recruited. We have isolated live CD14+ human monocytes from recruited PD, CD and control cases for *ex vivo* IFN-γ stimulation. We found that IFN-γ potently enhanced gene expression of pro-inflammatory molecules and LRRK2 itself. Interestingly, specific LRRK2 inhibitors (CZC-25146 and GSK-2578215A) show further enhanced the gene expression, whereas FK506, a NFAT inhibitor, suppressed all the gene induction. M2397 LRRK2 CD risk allele group show enhanced IFN-γ responses in CD but not in control cohort. Monocytes isolated from PD cases with G2019S and R144C LRRK2 mutations show significantly suppressed IFN-γ responses, whereas monocytes from sporadic PD cohort are similar to the ones from control cohort. Interestingly, none of M2397 allele homozygotes are present in the PD cases with G2019S or R144C LRRK2 mutation. These data demonstrate that PD-linked and CD-associated LRRK2 mutations are significant modifiers of innate immune response and potentially important for the immunopathogenesis of PD and CD.

Disclosures: **T. Ikezu:** None. **H. Asai:** None. **S. Ikezu:** None. **B. Wolozin:** None. **F. Farraye:** None. **Z. Wszolek:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.07/C63

Topic: C.03. Parkinson's Disease

Support: MJFF

Title: microRNAs as progression biomarkers for Parkinson's disease

Authors: *S. KHOO;
Grand Valley State Univ., Grand Rapids, MI

Abstract: Parkinson's disease is the second most common neurodegenerative disorder. There is no cure nor definitive diagnosis of this progressive disease. When patients are first diagnosed, they are usually in a moderately advanced stage, which is a major roadblock for early therapeutic intervention. Hence, development of objective biomarkers for diagnosis and progression is essential to impact management and treatment of this devastating disease. Previously, we identified a panel of microRNAs (miRNAs) from plasma that can differentiate PD from healthy controls. Here, we evaluate the miRNA expression changes in PD patients over time and assess their potential as biomarkers to track PD progression. 30 plasma samples (15 fast and 15 slow progressors) with baseline (0 mo.) and endpoints (6-23 mo.) were processed and quantitative real-time PCR was performed. We found a subset of miRNAs showing statistically significant difference between baselines and endpoints, as well as between slow and fast progressors. This preliminary results suggest that miRNAs have great potential to serve as progression biomarkers for PD. This research project is funded by the Michael J. Fox Foundation for Parkinson's Research using the DATATOP samples.

Disclosures: S. Khoo: None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.08/C64

Topic: C.03. Parkinson's Disease

Support: NIH U'01

Title: Examination of iron-management proteins in Parkinson's disease patients

Authors: *A. M. SNYDER[1], J. R. CONNOR[1], C. STETTER[2], L. KONG[2], M. M. LEWIS[3], X. HUANG[3];
[1]Neurosurgery, MC H110, [2]Publ. Hlth. Sci., [3]Neurol., Penn State Univ. Coll Med., Hershey, PA

Abstract: Increased iron deposition in the brains of patients with Parkinson's disease (PD) has long been reported, yet the role of iron in disease progression or the management of iron in the parkinsonian brain remains to be elucidated. Key proteins that work synergistically to regulate iron uptake and storage are transferrin and ferritin, respectively. Ferritin has the capability of sequestering 4500 atoms of iron and is comprised of H- and L- subunits. H-ferritin is if particular interest in Parkinson's disease because it converts bio-reactive ferrous iron to relatively inert ferric iron. Data from autopsy tissue suggest that H-ferritin levels are decreased in Parkinson's disease specimens, but H-ferritin levels in the central nervous system, as reflected by cerebrospinal fluid (CSF), or in the periphery are unknown in the living PD patient. The overall goal of our work is to establish a biomarker profile to better understand the role of iron in Parkinson's disease pathophysiology. We hypothesize that ferritin levels, particularly those of H-ferritin, will be informative in understanding disease progression. We examined proteins of iron management in serum and CSF from PD patients and controls. No differences were detected in serum transferrin or ferritin between PD and control groups. H-ferritin levels in serum or CSF were not changed when all PD patients were treated as one group. However, when PD patients were sub-divided into disease duration categories of less than one year, one to five years, five to ten years, and over ten years, an important correlation between serum and CSF H-ferritin levels emerged. Patients with disease duration less than one year reflected the positive correlation between serum and CSF H-ferritin levels that is observed in controls. The data from PD patients with disease duration of one to five years showed a negative correlation between H-ferritin in serum and CSF. Patients beyond five years did not show a correlation between serum and CSF H-ferritin levels. These data suggest that iron management is not causative of PD pathology but does signify a shift in iron homeostasis two to five years after PD diagnosis. Investigation is ongoing to determine if H-ferritin levels have prognostic value for determining rate and severity of disease progression.

Disclosures: A.M. Snyder: None. **J.R. Connor:** None. **C. Stetter:** None. **L. Kong:** None. **M.M. Lewis:** None. **X. Huang:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.09/C65

Topic: C.03. Parkinson's Disease

Support: NSFC

973 project of China

Title: Specific changes of serum proteins in Parkinson's disease patients

Authors: *X. WANG[1], Y. SUN[2], B. CHEN[3], W. LU[2];
[1]Inst. Materia Med., Beijing, China; [2]Inst. of Materia Medica, Beijing, China; [3]Xuanwu Hosp., Beijing, China

Abstract: Recently we demonstrated that the protein ITI-H4 (inter-alpha-trypsin heavy inhibitor) and Apo-A-IV were specific changed in the serum of Parkinson disease patients (Lu *et al*, *Plos One*, 2014). It was interesting that each protein was found existing two subunits in serum. The full size (120 kDa) ITI-H4 was increased and the fragmented ITI-H4 (35 kDa) was decreased in PD patients. Meanwhile, the subunit A of Apo-A-IV (46 kDa) was not changed in PD group, but the subunit B (26 kDa) was significantly decreased. Above data was obtained from 20 PD patients and 20 health people. In order to further demonstrate and validate the results, we observed the changes of both proteins in 180 PD patients and investigated the regulation of the two proteins in the present study. The blood samples were collected from PD patients aged 50 - 75 and they were diagnosed PD in 5 years. The control blood samples were collected from the health people with the same age range. The serum proteins of ITI- H4 and Apo-A-IV were semi-quantity determined by using western blot method. It was showed that the expressions of full-long ITI-H4 (120 kDa) increased 29% and the 35 kDa fragment was decreased 18% in PD group compared with control group. The ratio of 120 kDa/35 kDa was enhanced 68% in PD patients compared to control. The expression of subunit A (46 kDa) of Apo-A-IV was no difference between PD and control groups. However, the 26 kDa subunit (subunit B) was decreased by 48% in PD patients. Our results are accordant with our previous finding. The regulatory mechanisms of both proteins were also investigated in this study. The two proteins and the subunits might be used as blood biomarkers of PD. They are also important in studies of pathogenesis and the pathophysiology of PD.

Disclosures: X. Wang: None. **Y. Sun:** None. **B. Chen:** None. **W. Lu:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.10/C66

Topic: C.03. Parkinson's Disease

Support: AU forskningsfond NEURODIN Ideas Center

M. J. Fox Foundation for Parkinson's disease

Title: Study of the CD163 receptor in Parkinson's disease: A prospective Biomarker?

Authors: *M. ROMERO-RAMOS[1], K. SHRIVASTAVA[1], D. BERG[2], H. J. MØLLER[3]; [1]NEURODIN, Dept. Biomedicine, Aarhus Univ., Aarhus C, Denmark; [2]Dept. of Neurodegeneration, Ctr. of Neurology, Hertie Inst. for Clin. Brain Res., Tuebingen, Germany; [3]Clin. Biochem., Aarhus Univ. Hosp., Aarhus, Denmark

Abstract: Neuropathological processes in Parkinson's disease (PD) are related to abnormalities of the presynaptic protein alpha-synuclein (AS), whose aggregation leads to the formation of amyloid fibrils in Lewy bodies, the pathological hallmark of PD. CD163 is a scavenger receptor normally expressed on peripheral monocytes/macrophages and whose expression is altered upon inflammation. Shedding of the CD163 from the cell surface occurs in human monocytes/macrophages and produces a soluble CD163 (sCD163) plasma protein. This shedding process occurs, at least partly, through a TLR4/Adam17 mediated signal. Interestingly, a positive correlation is seen between the sCD163 plasma level and the severity of various pathologies. Previous studies from our group showed an increased infiltration in brain of CD163+ cells and that CD163+ monocyte/macrophages may be used as tool to achieve neuroprotection in the 6-OHDA rat PD model. We hypothesized that there are changes in peripheral CD163 population and/or shedding of the protein during PD. To address this, we are analyzing expression of CD163 in peripheral blood mononuclear cells by cytometry and the levels of sCD163 by ELISA in patient serum and CSF samples. So far, we have observed an increase in sCD163 in CSF and serum of PD patients when compared to healthy control group and there was a gender effect with females showing higher sCD163 than males. We are currently studying the CD163+ cell population in PD patients as well as the possible influence of AS in the CD163 expression and/or shedding. Our results aim to further evaluate the CD163 population as a therapeutic target in PD as well as to assess the potential value of the sCD163 as a putative biomarker for PD.

Disclosures: M. Romero-Ramos: None. **K. Shrivastava:** None. **D. Berg:** None. **H.J. Møller:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.11/C67

Topic: C.03. Parkinson's Disease

Support: U01 GM092655

Title: Gene expression profiling of anterior cingulate cortex from subjects with Lewy body dementia

Authors: *M. PIETRZAK[1,2], A. PAPP[1], A. CURTIS[1], M. KATAKI[3], D. SCHARRE[3], G. REMPALA[4], W. SADEE[1];
[1]Ctr. for Pharmacogenomics, Col. of Med., [2]Div. of Biostatistics, Col. of Publ. Hlth., [3]Dept. of Neurology, Col. of Med., The Ohio State Univ., Columbus, OH; [4]Mathematical Biosci. Inst., The Ohio State Univ. Columbus, Columbus, OH

Abstract: The biology underlying the development and progression of Dementia with Lewy Bodies (DLB) pathology remains unclear. In this work we have applied next generation sequencing to analyze gene expression patterns altered by DLB pathology in anterior cingulate cortex - a brain region affected by DLB pathology. Gene network analysis was performed to assess pathways and gene-gene interactions, relevant to DLB etiology. Analysis of RNA profiles, using Ampli-seq panel of 20812 genes with ion torrent sequencing, was performed in brain samples from 12 DLB subjects and 10 age-matched controls. Analysis of differential gene expression revealed 109 genes down-regulated and 218 up-regulated in DLB samples. These groups included genes associated with neurological disorders, inflammatory response and cell death, such as adenosine A2a receptor, transforming growth factor beta 1, and chemokine receptor 4. To identify gene clusters associated with the disease we used a systems-biology approach based on weighted gene co-expression network analysis. The analysis revealed differences in co-expression patterns of genes associated with processes and pathways relevant to DLB pathology such as axonal degeneration or neuroinflammation. Our study provides a comprehensive picture of gene co-expression patterns in DLB derived from transcriptional changes in a disease-affected region of the brain. These findings provide a framework for functional studies of gene-gene interactions in DLB.

Disclosures: M. Pietrzak: None. **A. Papp:** None. **A. Curtis:** None. **M. Kataki:** None. **D. Scharre:** None. **G. Rempala:** None. **W. Sadee:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.12/C68

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

Scåne University Hospital

Title: Angiogenic biomarkers in Parkinson's disease dementia: clinical-biochemical-pathological correlations

Authors: *V. FRANCARDO[1], S. JANELIDZE[2], G. SERRANO[3], T. BEACH[3], C. KONRADI[4], O. HANSSON[2,5], M. CENCI[1];

[1]Lund Univ., Lund, Sweden; [2]Dept. of Clin. Sci., Malmö Univ., Malmö, Sweden; [3]Banner Sun Hlth. Res. Inst., Sun City, AZ; [4]Dept. of Pharmacol., Vanderbilt Univ., Nashville, TN; [5]Memory Clinic, Skåne Univ. Hosp., Lund, Sweden

Abstract: Studies performed in a large number of neurological diseases have revealed an association between altered perfusion and maladaptive angiogenesis in disease-affected brain regions. This maladaptive response is usually accompanied by neuroinflammation, and may contribute to neurodegeneration. These processes have been well documented in Alzheimer´s disease, where cortical microvascular dysfunction is an early event. It is currently unknown whether similar processes accompany the development of dementia in Parkinson´s Disease (PD). This hypothesis seems compatible with the results of functional imaging studies, which have shown reductions in both local blood flow and glucose uptake in the cerebral cortex in in PD patients affected by dementia. The aim of this study was to explore the occurrence of maladaptive angiogenesis in PD dementia. To this end, we measured levels of angiogenic cytokines in samples of cerebrospinal fluid (CSF) from three different cohorts of PD patients, with or without dementia, and non-PD controls. We obtained post-mortem samples of temporal cortex (middle temporal gyrus) from one of these cohorts. In these samples, we examined the density of microvessels (immunostained for CD34) and their expression of nestin, a marker of reactive endothelium that is upregulated during active angiogenesis. On adjacent sections, we measured the total number of neurons (immunostained for NeuN). The results of CSF analysed showed significantly higher levels of Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF) in PD patients compared to controls, whether or not the patients had been affected by dementia. In brain cortical tissue, microvessel density was larger in PD patients with dementia than in the non-demented PD and control groups. There was also a trend towards nestin upregulation in the former group, which did not however reach significance. The counts of NeuN-positive cells (neurons) did not reveal any difference between the groups. The promising results obtained in this study call for further investigations on the role of cortical microvascular pathology and angiogenic responses in the development of PD dementia.

Disclosures: V. Francardo: None. **S. Janelidze:** None. **G. Serrano:** None. **T. Beach:** None. **C. Konradi:** None. **O. Hansson:** None. **M. Cenci:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.13/C69

Topic: C.03. Parkinson’s Disease

Title: Natural history of rigidity in Parkinson's disease

Authors: *R. XIA[1], A. MUTHUMANI[2], Z.-H. MAO[3], D. POWELL[4];
[1]Dept of Physical Therapy, Univ. of St. Mary, Leavenworth, KS; [2]Dept. of Engin., Montana State Univ., Bozeman, MT; [3]Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [4]Dept. of Physical Therapy, Campbell Univ., Buies Creek, NC

Abstract: Parkinson's disease (PD) is a chronic neurodegenerative disease with a relentlessly progressive course. Rigidity is one of the clinical hallmark symptoms associated with PD, and defined as an increased resistance to passive movement throughout an entire range of motion. Recent studies indicate that rigidity is the result of both increased muscle reflex responses (neural component) and altered mechanical properties of muscle fibers (non-neural component). Emerging evidence from clinical and imaging studies has suggested that rigidity progresses fastest among the hallmark symptoms of PD. The purpose of this study was to examine the relationship between disease history and the neural and non-neural contributions to rigidity in PD. Twelve subjects with idiopathic PD, with a disease duration from one to 13 years, participated in a study protocol, following an overnight withdrawal of dopamine-replacement therapy. Torque resistance of the more affected wrist joint was measured during passive flexion and extension movements in patterns of pseudorandom binary sequences. To differentiate the neural and non-neural components, a parallel-cascaded system identification technique was applied. Pearson correlation coefficients were calculated to quantify the correlations of disease duration with neural and non-neural contributions to rigidity, respectively. Relationship between subjects' age and rigidity was also evaluated. Analyses revealed significant correlations between disease history and neural rigidity ($r = 0.703$, $p = 0.01$) and between disease history and non-neural rigidity ($r = 0.735$, $p = 0.006$). No correlation was observed between age and either neural ($r = 0.243$) or non-neural ($r = 0.235$) rigidity. Our findings show that the longer the disease duration, the greater rigidity was, regardless of subject's age. Clinical Relevance: The quantitative evaluation obtained from this study supports the natural history of disease progression assessed by clinical observation. Non-pharmacologic therapeutic intervention such as manual stretching and relaxation technique shows potential in managing abnormal muscle tone. Future research is directed to determine whether such interventions are effective in managing the progression of parkinsonian rigidity.

Disclosures: **R. Xia:** None. **A. Muthumani:** None. **Z. Mao:** None. **D. Powell:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.14/C70

Topic: C.03. Parkinson's Disease

Support: HKU Seed Funding Program for Basic Research (201211159163)

Health and Medical Research Fund (01121636)

Title: Visuomotor control in patients with Parkinson's disease

Authors: *J. CHEN[1], S.-L. HO[2], M.-C. LEE[3,4], S.-K. CHANG[2], Y.-Y. PANG[2], L. LI[1]; [1]Dept. of Psychology, The Univ. of Hong K, Hong Kong, Hong Kong; [2]Div. of Neurology, Univ. Dept. of Medicine, The Univ. of Hong Kong, Hong Kong, Hong Kong; [3]Lab. of Neuropsychology, The Univ. of Hong Kong,, Hong Kong, Hong Kong; [4]The State Key Lab. of Brain and Cognitive Science, The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Previous studies have suggested that the deteriorated visuomotor control in patients with PD (Parkinson's disease) is due to deficits in various aspects of the sensory-motor processing rather than motor control itself. In the current study, by taking a control-theoretic approach, we systematically examined how PD and antiparkinsonian medication affect visuomotor control and the underlying sensory-motor system. We tested 20 PD patients in both ON and OFF medication states and 20 demographically matched healthy controls with a commonly used manual control task. Specifically, in each 95-s trial, participants were instructed to use a joystick to control a randomly moving target to keep it centered on a computer display. We found that although antiparkinsonian medication improved visuomotor control in PD patients, they still showed significantly decreased control precision (measured by RMS error) and response amplitude (gain) as well as increased response delay (phase lag) compared with healthy controls. Our model-driven analysis revealed that PD impairs the responsiveness and the predicting ability of the sensory-motor system as well as the stability of the neuromuscular system. Taking antiparkinsonian medication improves the responsiveness of the sensory-motor system. More importantly, it improves the ability of the sensory-motor system to use the target velocity information to predict the target position error and generate lead control ahead of time up to the level of healthy controls. However, taking antiparkinsonian medication does not improve the stability of the neuromuscular system. These results support the claim that the effect of antiparkinsonian medication on visuomotor control is mainly through improving visual-stimulus-dependent sensory-motor processing. The present study provides the first quantitative examination of the effects of PD and antiparkinsonian medication on the sensory-motor system underlying visuomotor control. The findings have practical implications for developing sensitive assessment tools to evaluate the efficacy of different therapies for PD and preliminary screening and training tools for fitness-to-drive in PD patients.

Disclosures: J. Chen: None. **S. Ho:** None. **M. Lee:** None. **S. Chang:** None. **Y. Pang:** None. **L. Li:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.15/C71

Topic: C.03. Parkinson's Disease

Title: Rhythmic auditory cues shape neural network plasticity underlying motor control in Parkinson's disease

Authors: ***M. H. THAUT**[1], K. BRAUNLICH[2], B. KLUGER[3], G. MCINTOSH[4], C. SEGER[2]; [1]Ctr. for Biomed. Res. in Music, Colorado State Univ., Fort Collins, CO; [2]COLORADO STATE UNIVERSITY, FORT COLLINS, CO; [3]UNIVERSITY OF COLORADO, DENVER, CO; [4]UNIVERSITY OF COLORADO HEALTH, FORT COLLINS, CO

Abstract: It is well established that rhythmic auditory cues can improve gait and other motor behaviors in Parkinson's disease (PD) and other disorders. However, the neural systems underlying this therapeutic effect are largely unknown. It is well known that the basal ganglia subserves important functions in rhythmic motor timing. However, since PD is characterized by basal ganglia dysfunction, the question arises how rhythmic auditory cues facilitate motor control in PD: putatively, by either maximizing residual basal ganglia function or by shaping a 'bypass' network, e.g., by involving cortico-cerebellar circuits. To investigate this question we scanned people with Parkinson's disease (Hoehn & Yahr level 3) and healthy controls using fMRI. Subjects performed a rhythmic motor behavior (right hand finger tapping) with and without simultaneous audiovisual rhythmic cues at two different speeds.In the Parkinson's group, the presence of simultaneous rhythmic cues during tapping was associated with greater activity in a large neural network including the anterior cingulate and medial frontal regions, bilateral middle frontal gyri, bilateral parietal lobe and insular cortices, and cerebellum and body of the caudate nucleus. A graph theoretical analysis showed a greater functional connectivity between motor regions (left pre and postcentral gyrus, SMA) and between these motor regions and the visual and auditory cortex in the Parkinson's group when simultaneous audiovisual rhythmic cues were present. We interpret our results as indicating that the temporal rhythmic sensory information is driving multiple compensatory mechanisms by shaping an 'assist' network for the basal ganglia consisting of increased activation in cortico-cerebellar regions. This 'assist' network was not seen in PD subjects tapping without rhythmic cues and in healthy subjects with and without rhythmic cuing.

Disclosures: **M.H. Thaut:** None. **K. Braunlich:** None. **B. Kluger:** None. **G. Mcintosh:** None. **C. Seger:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.16/C72

Topic: C.03. Parkinson's Disease

Title: BDNF-TrkB signaling in lymphocytes of patients with Parkinson's disease increases after a four-week intensive rehabilitation treatment

Authors: *C. FONTANESI[1,2,3], S. KVINT[3], G. FRAZZITTA[4], R. BERA[4], D. FERRAZZOLI[4], A. DI ROCCO[5], H. REBHOLZ[3], E. FRIEDMAN[3], G. PEZZOLI[6], A. QUARTARONE[3,7], H.-Y. WANG[3], M. F. GHILARDI[3];
[1]City Col. of New York, New York, NY; [2]Biol., The Grad. Center, CUNY, New York, NY; [3]CUNY Med. Sch., New York, NY; [4]Dept. of Parkinson Dis. Rehabil., "Moriggia Pelascini" Hosp., Gravedona ed Uniti, Italy; [5]NYU-Langone Sch. of Med., New York, NY; [6]Istituti Clinici di Perfezionamento, Milano, Italy; [7]Univ. of Messina, Messina, Italy

Abstract: In a combined animal and human study, we have previously shown that a five-day treatment that enhances cortical plasticity also facilitates brain-derived neurotrophic factor (BDNF)-tyrosine receptor kinase B (TrkB) signaling and increases activated TrkB and N-methyl-D-aspartate receptor (NMDAR) association in both the cortex and the peripheral lymphocytes. Patients with Parkinson's disease (PD) in general show decreased cortical plasticity, as demonstrated by electrophysiological and behavioral studies. Here we test the hypothesis that an exercise program that improves motor function and seems to slow down symptoms' progression can enhance BDNF-TrkB signaling in lymphocytes. Sixteen patients with PD underwent a four-week Multidisciplinary Intensive Rehabilitation Treatment (MIRT), which included aerobic training, physical and occupational therapy. Blood was collected before, after two- and four-week MIRT. Lymphocytes were isolated to examine BDNF-TrkB signaling induced by incubation with recombinant human BDNF. TrkB signaling complexes, extracellular-signal-regulated kinase-2 and protein-kinase-B were immunoprecipitated; content of immunocomplexes was determined by Western blotting. After MIRT, all patients showed improvement in motor function ($p< 0.01$) as shown in previous studies. TrkB interaction with NMDAR and BDNF-TrkB signaling increased in peripheral lymphocytes at receptor, intracellular mediators and downstream levels ($p< 0.001$). Moreover, the increases in BDNF-TrkB signaling and TrkB-NMDAR interaction were significantly correlated with the decrements in UPDRS II and total scores. We conclude that MIRT promotes changes of the immune function in PD. The reduced severity of PD symptoms together with enhanced lymphocyte BDNF-TrkB signaling further suggests that the immune system might play a role in neurorestoration and recovery of function. We finally speculate that, as BDNF-TrkB signaling in cortex and lymphocyte are partially correlated, MIRT might also increase cortical plasticity.

Disclosures: C. Fontanesi: None. **S. Kvint:** None. **G. Frazzitta:** None. **R. Bera:** None. **D. Ferrazzoli:** None. **A. Di Rocco:** None. **H. Rebholz:** None. **E. Friedman:** None. **G. Pezzoli:** None. **A. Quartarone:** None. **H. Wang:** None. **M.F. Ghilardi:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.17/C73

Topic: C.03. Parkinson's Disease

Title: Evaluation of anti-alpha-synuclein autoantibody affinity in Parkinson's disease and multiple system atrophy

Authors: *T. BRUDEK[1,2], J. FOLKE[1], K. WINGE[3,2], K. FOG[4], B. PAKKENBERG[1], L. ØSTERGAARD PEDERSEN[4];
[1]Res. Lab. For Stereology and Neuroscienc, Copenhagen, Denmark; [2]Bispebjerg Movement Disorders Biobank, Copenhagen, Denmark; [3]Dept. of Neurol., Bispebjerg and Frederiksberg Hosp., Copenhagen, Denmark; [4]H. Lundbeck A/S, Valby, Denmark

Abstract: Objectives: To investigate the properties of naturally occurring anti-α-syn antibodies in plasma from patients with Parkinson's Disease (PD) and Multiple System Atrophy (MSA). Background: PD and MSA are progressive brain disorders caused by loss of nerve cells in specific areas of the brain. This loss causes problems with movement, balance and/or autonomic functions of the body. Compared to PD patients, patients with MSA have more widespread brain pathology, shorter survival, and poor treatment response. Aggregation of filamentous α-synuclein (α-syn) is thought to play a key role in both disorders. Naturally occurring autoantibodies (NAbs) probably act in eliminating circulating proteins, before they can elicit a damaging response. Presence, but not specificity (our pilot study) of anti-α-syn NAbs has previously been repeatedly investigated in PD. However, the reported level differences in plasma-borne NAbs directed against the putatively most important self-antigen in PD and MSA, α-syn, have been inconsistent. Thus, little is known still about the specific properties of anti-α-syn NAbs in PD and MSA. Methods: We have investigated the affinity of anti-α-syn NAbs in plasma samples from 46 PD patients, 18 MSA patients and 41 age-matched normal controls using competitive enzyme-linked immunosorbent assay (ELISA) set-up. The key event of a competitive reaction results from occupation of antibody binding sites with increasing concentrations of free antigen (α-syn) and a subsequent measurement of free antibodies on plates with immobilized antigen (α-syn). Moreover, using similar competition assay, we assess cross-reactivity of the anti-α-syn NAbs toward β-, and γ-synucleins in MSA and PD vs healthy controls. Results: Our data indicate that the frequency of PD patients with high affinity phenotype is lower compared to healthy controls, whereas MSA patients practically do not express the high affinity phenotype of plasma anti-α-syn antibodies. The preliminary data from the cross reactivity assay suggest declined cross reactivity of anti-α, β-, and γ-syn NAbs in both neurodegenerative disorders. Conclusions: Our results indicate that the PD and MSA patients have lower titer of high-affinity anti-α-syn antibodies and thus lower ability for immune clearance of toxic α-syn species/oligomers. This affinity aberration may be a new mechanism of impaired clearance of cerebral α-syn and have important implications for the development of immune-based therapeutic strategies.

Disclosures: **T. Brudek:** None. **J. Folke:** None. **K. Winge:** None. **K. Fog:** None. **B. Pakkenberg:** None. **L. Østergaard Pedersen:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.18/C74

Topic: C.03. Parkinson's Disease

Support: R01 NS064934

U18NS082132

Title: Deregulation of HNF4A and PTBP1 in Parkinson's disease

Authors: ***C. WAN**[1], A. B. WEST[2], A. B. RAWLINS[3];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [3]Neurol., [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Parkinson's disease (PD) affects more than one million Americans, not excluding those that have yet to be officially diagnosed. As the number of PD patients increases from year to year, efforts to find biological markers that precede the clinical onset of PD have increased, so that preventative therapies for patients in the earlier stages of the disease may be implemented. Previously published studies, using a small sample size with limited clinical phenotyping, have indicated that the up-regulation of hepatocyte nuclear factor 4 alpha (HNF4A), part of the nuclear receptor family of transcription factors, and the down-regulation of polypyrimidine tract binding protein 1 (PTBP1), which functions in mRNA processing, may be exaggerated in the blood of PD patients (Santiago and Potashkin, PNAS 2015). Through quantitative PCR assays, we compare the levels of HNF4A and PTBP1 in blood collected in the Parkinson Disease Biomarkers Program (PDPB). Our study involves 200 PD patients and 200 healthy controls, matched for race, ethnicity, age, and gender. We further investigate the change in regulation of the two genes in correlation to PD severity and other clinical metrics associated with the extensive phenotyping in the PDBP. These data will help establish whether HNF4A and PTBP1 might be useful in the diagnosis and prognosis of Parkinson disease.

Disclosures: **C. Wan:** None. **A.B. West:** None. **A.B. Rawlins:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.19/C75

Topic: C.03. Parkinson's Disease

Support: CDCH grant M-1019-11-03C

Title: Increase of red blood cell putrescine detected by cze-lifd in Parkinson's patients

Authors: L. R. BETANCOURT, P. V. RADA, G. A. CEBALLOS, H. F. ARAUJO, *L. F. HERNANDEZ;
Univ. de los Andes, Merida, Venezuela, Bolivarian Republic of

Abstract: Polyamines are modulators of cell functions such as cell proliferation and differentiation and they are involved in many pathological conditions associated to neurodegenerative diseases. Alterations in the expression and activity of polyamine metabolic enzymes have been reported in schizophrenia, affective disorders, anxiety, suicidal behavior and Parkinson disease (PD).We report a new technique to measure putrescina based on CZE-LIFD and an increase of blood putrescine in PD patients. We took a 2.8 micromolar solution of thiocarbamyl-putrescine and diluted it ten times in 20 mM carbonate buffer to obtain 1.4 μM, 0.7 μM, 350 nM, 175 nM, 87.5 nM, 43.7 nM, 21 nM, 10 nM and 5 nM concentrations. The standards and the samples were run in a fused silica, 25 micrometers ID capillary filled with 40 mM Sodium Dodecyl Sulphate and 20 mM Sodium Tetraborate buffer. There was a linear relation between concentration and signal amplitude in the whole range of concentrations (R= 0.998) and in the 5 lower concentrations (R= 0.995). The lowest concentration (5 nanoM) produced a signal to noise ratio of 10:1. We found a significant increase of putrescine in the red blood cells and a non-significant increase of putrescine in plasma of PD patients as compared to matched control ($p<0.04$ and $p<.14$) respectively. These findings support a pathophysiological mechanism for PD and have great potential to provide a marker of PD.

Disclosures: L.R. Betancourt: None. **P.V. Rada:** None. **G.A. Ceballos:** None. **H.F. Araujo:** None. **L.F. Hernandez:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.20/C76

Topic: C.03. Parkinson's Disease

Support: NIH Grant NW-085331

NIH Grant T32 HD-057845

NIH Grant T32 EB-009406

Craig H. Nielsen Foundation Grant 260215

Northwestern Memorial Foundation Parkinson's Disease & Movement Disorders Advisory Council Grant

Title: Paradoxical changes in intrinsic motoneuron excitability between flexors and extensors in Parkinson's disease

Authors: *J. M. WILSON[1], C. K. THOMPSON[2], L. C. MILLER[1], C. MACKINNON[3], C. J. HECKMAN[2];
[1]Physical Therapy & Human Movement Sci., [2]Physiol., Northwestern Univ., Chicago, IL;
[3]Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Alpha-motoneurons (MNs) possess active membrane properties that significantly influence their capacity for synaptic amplification and self-sustained firing. These properties are facilitated by persistent inward sodium and calcium currents (PICs) in the MNs that are principally dependent on the presence of serotonin and norepinephrine from the brainstem. In Parkinson's disease (PD), degeneration of the caudal raphe nuclei and the locus coeruleus occur relatively early in the disease, suggesting a loss of serotonergic and noradrenergic drive to the spinal cord may contribute to observed abnormalities in muscle activation patterns via alterations in PICs and subsequent changes in MN excitability. In addition, individuals with PD show greater strength deficits and EMG abnormalities in extensors over flexors, suggesting that a loss of MN excitability may be greater in extensor MNs than flexor MNs. We assessed MN intrinsic excitability in upper limb flexors and extensors of mild-moderate PD patients (OFF-state) and healthy controls by recording spike trains of MNs during isometric trapezoid contractions to 10% MVC. We then used a lower-threshold "reporter" unit as an index of descending common drive to a higher-threshold "test" unit, deriving a value known as the delta-F as an estimate of intrinsic excitability of the test unit (Gorassini et al. 2002). As expected, we found that the delta-F in PD was significantly lower than controls in extensors, but were surprised to find that the delta-F in flexors was nearly two-fold higher in PD compared to controls. PD patients also exhibited a greater occurrence of self-sustained firing behaviors indicative of increased PICs. Possible reasons for these results are discussed, including the potential role of reciprocal inhibition and the possibility of constitutively-active PICs in the absence of monoaminergic drive. The present work provides evidence that spinal MNs may actively contribute to PD symptoms via a loss of non-dopaminergic spinal neuromodulators, thus reframing PD motor deficits as having both supraspinal and spinal components.

Disclosures: J.M. Wilson: None. **C.K. Thompson:** None. **L.C. Miller:** None. **C. MacKinnon:** None. **C.J. Heckman:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.21/C77

Topic: C.03. Parkinson's Disease

Support: German Research Foundation (KFO 219)

Koeln Fortune Program

German Research Foundation (Transregional Collaborative Research Center 134)

Title: Bimanual synchronized finger tapping in young and elderly subjects

Authors: *F. JUNG[1], P. LÖHRER[1], I. WEBER[1], F. NETTERSHEIM[1], T. A. DEMBEK[1], E. A. PELZER[2], C. HUBER[1], M. TITTGEMEYER[2], L. TIMMERMANN[1];
[1]Univ. Hosp. Cologne, Depart. of Neurol., Cologne, Germany; [2]Max Planck Inst. for Metabolism Res., Cologne, Germany

Abstract: Coordination of bimanual hand and finger movements is crucial for daily activities and controlled by the supplementary motor area, the premotor and prefrontal cortex. Even though brain areas responsible for the execution of bimanual movements are largely discovered, their causal interplay is not well understood. Apart from a general investigation of hierarchical coupling within a core motor network, we investigated changes occurring during physiological aging since motor functions are known to decline with age. While in general, elderly subjects show an age-related slowing and decrease of performance accuracy, they were shown to perform equally well as young subjects during temporally synchronized, "in-phase" movements yet exhibiting problems with the execution of unsnchronized „anti-phase" movement. In order to unravel these age-related changes in bimanual coordination and potential differences in coupling of brain areas, a complex "anti-phase" finger tapping task was designed. Subjects learned a sequence of finger movements that should be executed while, at the same time, the other hand was used to tap a different sequence. Simultaneously, a surface EEG with 128 electrodes from 23 young (mean age: 25 ± 2) and 28 elderly (mean age: 61 ± 7) subjects was recorded. Behavioral analysis demonstrated that elderly subjects made significantly more mistakes (mean old: 34.36 %; mean young: 20.46 %, $p = 0.003$) and needed more time (mean old: 3.19 s; mean young: 2.27 s, $p < 0.001$) than young subjects. Additionally, the analysis of electrophysiological data revealed differences in event-related potentials (ERPs) averaged time-locked to the "go" stimulus: If the learned rule was performed with the right, i.e. the dominant hand, ERPs for young and old subjects differed between 500 and 750 ms after stimulus onset, with old subjects showing more positive potentials. Significant differences between both groups were found mainly on the right hemisphere above the postcentral sulcus and the angular gyrus. Preliminary analysis showed that ageing not only affects behavior with respect to changes in a general speed-accuracy tradeoff but also electrophysiological processing during bimanual coordination. A successive network analysis using Dynamic causal modelling (DCM) for evoked responses will uncover differences

in the coupling of brain areas between young and elderly subjects and enable us to better understand physiological changes occurring in the aging brain.

Disclosures: F. Jung: None. **P. Löhrer:** None. **I. Weber:** None. **F. Nettersheim:** None. **T.A. Dembek:** None. **E.A. Pelzer:** None. **C. Huber:** None. **M. Tittgemeyer:** None. **L. Timmermann:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.22/C78

Topic: C.03. Parkinson's Disease

Title: Repetitive finger movement and quality of life in persons with Parkinson's disease

Authors: *J. UZOCHUKWU, R. WILLE, E. L. STEGEMÖLLER;
Iowa State Univ., Ames, IA

Abstract: The performance of repetitive finger movements is a clinical tool used to assess the severity, progression, and treatment efficacy of Parkinson's disease (PD). Studies have shown that when some persons with PD performed acoustically cued repetitive finger movements at rates near to and above 2 Hz, participants exhibited increased movement rate, reduced movement amplitude, and loss of phase accompanied by frequent hesitations. The effect of this movement impairment on quality of life (QOL) of people with PD is unknown. As such, the purpose of this study was to examine the QOL ratings of people with PD who demonstrate impairment of repetitive finger movement compared to those who do not. Fifty-one participants (mean age = 69 ± 10; 24 male, 27 female) with PD completed an acoustically cued repetitive finger movement task. The acoustic cue incremented from a rate of 1 Hz to 3 Hz in 0.25 Hz. Participants also completed the Parkinson's Disease Questionnaire - 39 (PDQ-39) as a measure of QOL. Based upon performance on the repetitive movement task (differences in movement rate and movement amplitude), participants were divided into groups of with and without impairment. Group differences were analyzed across individual domains on the PDQ - 39. Results revealed no significant differences between groups on index and individual domain scores on the PDQ - 39. However, those with repetitive finger movement impairment showed a trend of having higher scores across all domains of the PDQ - 39 compared to the non-impaired group. The higher scores were indicative of poorer QOL. These results suggest that repetitive finger movement impairment has a negative impact on perceived QOL in people with PD. A larger sample size of participants who demonstrate this impairment is needed to fully characterize differences among groups of people with PD that demonstrate repetitive finger movement impairment and those that do not.

Disclosures: **J. Uzochukwu:** None. **R. Wille:** None. **E.L. Stegemöller:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.23/C79

Topic: C.03. Parkinson's Disease

Support: CIHR grant MOP-114916

Title: The implication of basal ganglia α7 nicotinic acethylcholine receptors (nAChr) in MPTP-lesioned monkeys and Parkinsonian patients with motor complications

Authors: N. MORIN[1], M. MORISSETTE[1], L. GRÉGOIRE[1], A. RAJPUT[2], A. H. RAJPUT[2], *T. P. DIPAOLO[1];
[1]Ctr. de Recherche de CHUQ-CHUL, Quebec, QC, Canada; [2]Div. of Neurol., Univ. of Saskatchewan, Royal Univ. Hosp., Saskatoon, SK, Canada

Abstract: In Parkinson's disease levodopa (L-Dopa) is the main symptomatic therapy to alleviate basal ganglia dysfunctions. However, chronic L-Dopa administration leads to motor complications, including L-Dopa-induced dyskinesias (LID) that represent a major therapeutic challenge in the management of Parkinson's disease treatment. The ability of nicotine and nicotinic receptor agonists to alleviate LID in parkinsonian animal models suggests an important role of the cholinergic system in the pathophysiology of LID. The most common subtypes of nicotinic acethylcholine receptors (nAChr) in the striatum are α4β2, α6β2 and α7. The α4β2 and α6β2 nAChr subtypes are reported to be implicated in the reduction LID and recent evidences support also an implication of the α7 nAChr subtype in LID. Indeed, we have shown that an α7 nAChr partial agonist reduced the intensity of LID in monkeys lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In the present study, we investigated α7 nAChr specific binding by autoradiography, with the ligand [125I]α-bungarotoxin, in post-mortem brains of parkinsonian patients and MPTP monkeys. MPTP monkeys were treated for one month with L-Dopa and developed LID while those treated with L-DOPA and MPEP, a specific antagonist of the metabotropic glutamate 5 (mGlu5) receptor, developed significantly less intense LID. Normal control and saline-treated MPTP monkeys were also included in the study. In the brains of parkinsonian patients who developed LID, α7 nAChr specific binding was elevated in the caudate nucleus and putamen while it was unchanged in the two segments of the globus pallidus. In patients without motor complications, α7 nAChr specific binding was elevated only in the caudate nucleus. Moreover, in parkinsonian patients with no LID but who experienced wearing-off, α7 nAChr specific binding in the caudate nucleus and putamen was lower than was observed in patients with LID. In monkeys, nAChr specific binding was elevated in the caudate nucleus and putamen as well as in the globus pallidus of dyskinetic monkeys as compared to controls and

MPTP monkeys treated with saline. Interestingly, MPTP monkeys treated with L-Dopa and MPEP had their nAChr specific binding similar to those in intact control animals. Moreover, positive linear correlations between dyskinesia scores of the monkeys and their [125I]α-bungarotoxin specific binding levels were observed in the caudate nucleus, putamen, and the globus pallidus. These findings support a possible involvement of α7 nAChr in LID and targeting this receptor could be an interesting therapeutic strategy to alleviate motor complications in Parkinson's disease.

Disclosures: N. Morin: None. **M. Morissette:** None. **L. Grégoire:** None. **A. Rajput:** None. **A.H. Rajput:** None. **T.P. DiPaolo:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.24/C80

Topic: C.03. Parkinson's Disease

Title: Human dopaminergic neurons used in an *in vitro* model of Parkinson's disease

Authors: *M. ROACH[1], K. GOMES[1], R. MALAVARCA[1], K. COOK[2], S. CHVATAL[2]; [1]PhoenixSongs Biologicals, Inc., Branford, CT; [2]Axion BioSystems, Atlanta, GA

Abstract: Parkinson's Disease (PD) is a chronic neurodegenerative disease, affecting approximately 1 million individuals in the US alone which is more than people diagnosed with multiple sclerosis, muscular dystrophy and Lou Gehrig's disease combined.. Parkinson's disease results in the loss of dopamine neurons (DA-neurons) that innervate the striatum. These dopamine neurons regulate the activity of a cortico-striatal-pallidal-thalamo-cortical (basal ganglia) circuit that controls the initiation and execution of motor and cognitive patterns. The loss of dopaminergic regulation of this circuit accounts for the development of the cardinal motor symptoms of the disease as well as many of the cognitive sequelae. Currently, a robust *in vitro* model of functional DA-neurons does not exist although many laboratories are trying to develop one. Therefore we set out to develop a robust *in vitro* Parkinson's model using stem cell-derived dopaminergic neurons at a scale that will be compatible with high throughput screens (HTS). Here we report development of a new PD model using human neural stem cells lineage committed to differentiate into a neural population where >60% of the neurons are TH+ mature functional DA-neurons that remain functional beyond 60 days in culture. Data reported will include standard MPP+ assays and neural network activity measured on multi-electrode arrays MEAs.

Disclosures: M. Roach: None. **K. Gomes:** None. **R. Malavarca:** None. **K. Cook:** None. **S. Chvatal:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.25/C81

Topic: C.03. Parkinson's Disease

Support: NIH CTSA UL1TR000117

Title: Using a modified cannula delivery system to implant sural nerve grafts into the rhesus macaque midbrain

Authors: J. E. QUINTERO[1,2], E. S. FORMAN[2], Y. AI[2], A. K. EVANS[2], R. M. WEEKS[2], F. POMERLEAU[2], P. HUETTL[2], *L. H. BRADLEY[3], R. GRONDIN[2], Z. ZHANG[2], G. GERHARDT[1,2,4], C. G. VAN HORNE[1,2,4];
[1]Brain Restoration Ctr., [2]Anat. & Neurobio., [3]Anat. and Neurobio., [4]Neurosurg., Univ. of Kentucky, Lexington, KY

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a loss of dopaminergic function. There is currently no effective treatment to slow or prevent its progression. Studies have shown that neurotrophic factors can promote dopaminergic function in areas like the substantia nigra, which is affected in PD. It has also been shown that Schwann cells in peripheral nerves might be a source of growth factors, including GDNF, NDF, BDNF, and NT-3. An FDA-approved Phase I clinical trial is currently ongoing at the University of Kentucky to assess the safety and efficacy of implanting an autologous sural nerve graft into the substantia nigra of patients with PD. While functional improvements have been seen in these participants post-implantation, how the sural nerve graft interacts with the surrounding brain tissue is unclear. To address this knowledge gap, similar procedures were performed in two normal, adult female rhesus macaques to study the histological and neurochemical effects from the implanted nerve grafts into the substantia nigra. A modified cannula/stylet assembly and modified Nexdrive system were implemented. First, the tip of a stainless steel 18G cannula/stylet was cut to have a tapered blunt end. Then, a 1 x 5mm side window was created, 4mm from the cannula tip to load the sural nerve tissue. Next, a Nexdrive system was adapted to hold the cannula while allowing both the cannula and stylet to be individually locked down for insertion into the brain parenchyma. MRI-guided sural nerve grafts were performed in both animals without post-surgical complications. Animals were monitored for 8 weeks post-implant for changes in motor function and/or body weight, at which point they were necropsied and brain tissue collected for analysis. No significant changes in body weight or locomotor activity were observed over the course of the study. Histological analyses indicated sural nerve tissue delivery to the substantia nigra with tyrosine hydroxylase (TH) immunoreactive cells innervating the graft. Neurochemical analyses showed that in the ipsilateral side to the graft, dopamine content in the caudate was 21400 ± 1290 ng/g and in the putamen was 19800 ± 4660 ng/g while in the

contralateral side the caudate was 12400 ± 2180 ng/g and in the putamen was 12800 ± 2950 ng/g (mean ± SD). We conclude that our modified surgical hardware can be safely used to successfully deliver sural nerve tissue to the rhesus midbrain to further understand the associated mechanisms of action and support further clinical development of this promising therapy.

Disclosures: J.E. Quintero: None. **E.S. Forman:** None. **Y. Ai:** None. **A.K. Evans:** None. **R.M. Weeks:** None. **F. Pomerleau:** None. **P. Huettl:** None. **L.H. Bradley:** None. **R. Grondin:** None. **Z. Zhang:** None. **G. Gerhardt:** None. **C.G. van Horne:** None.

Poster

301. Alpha-Synuclein, LRRK2, and Other Molecular Mechanisms: Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 301.26/C82

Topic: C.03. Parkinson's Disease

Support: Arizona Center for the Biology of Complex Diseases Grant

University of Arizona Intramural Funds

Title: Analysis of cellular morphology, growth dynamics and autophagic mechanisms in primary skin fibroblasts from individuals diagnosed with sporadic Parkinson's disease

Authors: *A. J. FLORES[1,2], M. J. CORENBLUM[2], C. CURIEL[3], S. J. SHERMAN[2], L. MADHAVAN[2,4];
[1]Grad. Interdisciplinary Program in Physiological Sci., [2]Dept. of Neurol., [3]Arizona Cancer Ctr., [4]Evelyn F. McKnight Brain Inst., Univ. of Arizona, Tucson, AZ

Abstract: Human primary skin fibroblasts are easily accessible peripheral cells that have Parkinson's disease (PD)-relevant biochemical and gene expression profiles, and constitute a system which reflects the chronological and genetic aging of patients to form a patient-specific model of the disease. Recent data suggest that cytoskeletal and metabolic alterations play a role in the PD degenerative process. Because cytoskeletal and metabolic dynamics are also important determinants of cellular morphology, we systematically analyzed the morphological features and growth dynamics of primary fibroblasts generated from skin biopsies of individuals diagnosed with late-onset PD, as well as age-matched control subjects. Under phase contrast conditions, control and PD fibroblasts apparently differed with respect to spatial growth patterns, and cell size and shape. Therefore, to clearly visualize morphology, dermal fibroblasts were stained with a fluorescent phalloidin F-actin probe, and analyzed for five different shape and size parameters using CellProfiler software. In particular, comparisons of cell area, perimeter and minimum and maximum diameters revealed that fibroblasts from PD patients were significantly smaller than control fibroblasts. Additionally, PD fibroblasts displayed a significantly higher form factor, indicating that they were more circular than control fibroblasts. In terms of growth dynamics,

comparisons between control and PD cell lines showed no significant differences in cell viability, time to reach 90% confluence, nor in total cell count at 90% confluence. However, a trend towards higher cell count and greater time to reach 90% confluence, was noted in the PD fibroblasts. Additionally, immunocytochemical analysis revealed that fibroblasts from Parkinson's subjects had significantly higher expression of the PD-relevant protein alpha-synuclein (α-synuclein) than control fibroblasts, as reflected by higher intensity of α-synunclein staining per cell. Given that α-synuclein is degraded via chaperone-mediated autophagy (CMA, a selective form of autophagy), and also that aberrant α-synuclein may act as a CMA inhibitor, as a next step, we have begun to analyze autophagy-related mechanisms, in the patient fibroblasts. These studies provide a foundation for investigating PD-relevant structural and autophagic alterations in a patient-specific manner, and will complement future assessments in induced pluripotent stem cell (iPSC)-derived midbrain dopaminergic neurons generated from skin fibroblasts of PD subjects.

Disclosures: **A.J. Flores:** None. **M.J. Corenblum:** None. **C. Curiel:** None. **S.J. Sherman:** None. **L. Madhavan:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.01/C83

Topic: C.03. Parkinson's Disease

Support: BIBS/NPNI New Frontiers Award

NSF CBET-1402803

NSF CBET-1264816

Title: Investigating cortico-basal-ganglia neural dynamics with optogenetic stimulation in hemi-Parkinsonian rats

Authors: Z. YU[1], R. DARIE[1], J. PERGE[2], W. ASAAD[2,3], A. V. NURMIKKO[1], *I. OZDEN[1]; [1]Sch. of Engin., [2]Dept. of Neurosci., Brown Univ., Providence, RI; [3]Deparment of Neurosurg., Brown Univ. Alpert Med. Sch. and Rhode Island Hosp., Providence, RI

Abstract: Deep brain electrical stimulation (DBS) is an effective treatment for severe Parkinson's disease (PD), While DBS leads to alleviation of PD motor symptoms and suppression of abnormal beta oscillations (13-30 Hz) in the cortico-basal-ganglia system (CBGS), some motor deficits typically persist. Ongoing efforts to improve the efficacy of DBS are partly hampered by an incomplete understanding of the mechanisms of DBS and by the ambiguities associated with the effect of electrical stimulation. Optogenetics, with its cellular

specificity, offers a unique opportunity to investigate PD pathophysiology and to develop a more potent alternative to electrical DBS. Accordingly, we aim to investigate neural mechanisms of therapeutic action of electrical and optogenetic DBS by using microelectrode array (MEA)-based recordings of motor cortical activity, together with simultaneous recordings from and optogenetic stimulation at deep brain nuclei (e.g. subthalamic nucleus (STN) and globus pallidus (GP)). Here we present our preliminary data based on 6-OHDA induced hemi-Parkinsonian (hemi-PD) rats. We surveyed four behavioral paradigms, namely adjusting step (AS) test, amphetamine induced rotation (AIR), harnessed treadmill running (HTR), and limb use asymmetry (LUA) to quantify the extent of hemi-PD behavior. Our data confirms presence of motor deficits such as akinesia, LUA and AIR bias in hemi-PD rats. Electrical DBS of STN improved these motor deficits to some extent (reduced AIRs and increased number of steps in HTR), but not completely. Based on existing hypotheses about sources of abnormal activities in the CBGS, we tested potential target sites for optogenetic DBS. As a first target, optogenetic suppression of STN neurons through a step-function inhibitory virus, SwiChRCA, showed similar therapeutic effects as to the case of electrical DBS (drop in AIRs). Other target sites for optogenetic DBS include external and internal globus pallidi. To characterize the neural mechanisms of these interventions, we implanted 6x6 MEAs into the normal and lesioned motor cortices of hemi-PD rats. Our preliminary data confirmed presence of excess beta oscillations in the lesioned-side motor cortex. However, the oscillations were not synchronized throughout the whole motor cortex but intermittently appeared at different locations at different times. As various optogenetic stimulation sites might impact motor cortical activity and behavior differently, we will aim to develop a temporally coordinated multisite stimulation paradigm for constructively integrating the specific therapeutic effects of each type of stimuli.

Disclosures: **Z. Yu:** None. **R. Darie:** None. **J. Perge:** None. **W. Asaad:** None. **A.V. Nurmikko:** None. **I. Ozden:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.02/C84

Topic: C.03. Parkinson’s Disease

Support: Wallenberg Academy Fellowship to G.S.

ERC starting grant to G.S. ("SENSTRIATUM" 282012

Title: Sensory integration in the dopamine-depleted striatum

Authors: *M. KETZEF, A. BONITO-OLIVA, G. SPIGOLON, G. FISONE, G. SILBERBERG;
Karolinska Inst., Stockholm, Sweden

Abstract: Parkinson's disease (PD) is primarily characterized as a movement disorder, resulting from the loss of dopaminergic innervation particularly to the striatum (ST). PD patients and model animals often exhibit sensory impairments alongside with motor symptoms, yet little is known about them. We characterized the bilateral sensory integration in the dopamine (DA) depleted ST using in-vivo whole-cell patch-clamp recordings in anaesthetized mice, unilaterally injected with 6-hydroxydopamine in the medial forebrain bundle (MFB). Recordings were obtained from the striatum of the lesioned hemisphere during spontaneous activity and during ipsi- and contralateral whisker deflection. Direct and indirect pathway medium spiny neurons (MSNs) were identified either by co-localizing labeled neurons in D1/D2-GFP mice post-hoc, or by "online" optogenetic stimulation using an "optopatcher" during whole-cell recordings. In DA depleted animals, direct pathway (D1 expressing) MSNs responses to contralateral and bilateral whisker deflection were reduced in amplitude and rising slope and had delayed peak latency compared to controls. Delayed and reduced contralateral responses thus appeared more similar to ipsilateral ones, thereby rendering D1 MSNs indistinguishable from D2 MSNs. Chronic levodopa administration in lesioned mice recovered D1 MSNs responses to contralateral stimulation. Our results suggest that sensory deficits following DA depletion are caused primarily by selective attenuation of tactile responses in direct pathway MSNs.

Disclosures: M. Ketzef: None. **A. Bonito-Oliva:** None. **G. Spigolon:** None. **G. Fisone:** None. **G. Silberberg:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.03/C85

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS040894

NIH R01 NS079312

Title: Beta frequency oscillations are neither necessary nor sufficient for emergence of Parkinsonian symptoms in rats

Authors: *C. BEHREND[1,2], D. T. BROCKER[1], W. M. GRILL[1,3,4];
[1]Biomed. Engin., [2]Sch. of Med., [3]Neurobio., [4]Surgery, Duke Univ., Durham, NC

Abstract: In studying the pathophysiological mechanisms of Parkinson's disease (PD) a particular focus has been placed on determining the significance of synchronized oscillatory burst firing at frequencies of 13-30Hz (termed "beta band"). Correlative evidence has suggested a relationship between beta band oscillations observed in local field potentials (LFPs) and symptoms of bradykinesia and akinesia. We tested the hypothesis that a causal relationship exists between synchronized, beta frequency oscillations in cortical-basal ganglia circuits and the development of bradykinesia/akinesia in PD. We designed novel temporal patterns of deep brain stimulation to mimic the oscillatory bursting activity in the beta frequency band observed in parkinsonian patients and rats. First, we applied these beta frequency patterned stimulus trains (BPTs), along with continuous frequency controls, to a model of the intact basal ganglia to quantify the effects on simulated neural activity. BPTs preferentially increased beta band oscillatory power in model GPi neuron activity as compared to continuous low and high frequency controls. Second, we applied BPTs and continuous frequency controls unilaterally to the subthalamic nucleus (STN) in intact rats using implanted stimulating electrodes and measured the effects on akinesia using the bar test. We also recorded electrocorticograms (ECoG) from primary motor cortex (M1) and LFPs from globus pallidus (GP) to determine the effects of STN stimulation on neural activity. All stimulation patterns evoked stereotyped, polyphasic potentials in M1 and GP at the burst or continuous frequency, implying an increase in signal power generated by stimulation. However, no significant difference was found in length of time on the bar among stimulation patterns or compared to no stimulation controls. We repeated these experiments after administration of raclopride, a selective antagonist of D2 receptors. Raclopride administration alone resulted in a statistically significant difference in length of time spent on the bar but did not enhance any stimulation pattern specific differences in bar test performance. Neural recordings pre- and post-raclopride administration revealed no significant difference in the total percent of spectral power within the beta band in either M1 ECoG or STN LFPs. Taken together, these results suggest that beta frequency oscillations may not be necessary or sufficient for the generation of symptoms of bradykinesia/akinesia in PD.

Disclosures: **C. Behrend:** None. **D.T. Brocker:** None. **W.M. Grill:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.04/C86

Topic: C.03. Parkinson's Disease

Support: ANR 2010-1416-02

France Parkinson

CNRS

Aix-Marseille University

Title: Motor and cognitive functions of striatal cholinergic interneurons in Parkinson's disease: optogenetic and pharmacological approaches

Authors: ***S. ZTAOU**[1], M. LIBERGE[1], N. MAURICE[2], F. JOUEN[2], L. KERKERIAN-LEGOFF[2], C. BEURRIER[2], M. AMALRIC[1];
[1]Aix Marseille Univ. - CNRS UMR 7291, Marseille Cedex 03, France; [2]Aix-Marseille University, CNRS UMR7288, Marseille cdx3, France

Abstract: Disturbance in the central muscarinic cholinergic system has been implicated in several neurodegenerative pathology. In Parkinson's disease (PD), anticholinergic (ACh) drugs were the first widely accepted drugs before the discovery of L-DOPA. Their precise mechanism of action is still not clear, although it is believed that they work by correcting the imbalance between striatal dopamine and acetylcholine activity. Here, we examined the involvement of striatal ACh interneurons in motor and cognitive functions by manipulation of striatal cholinergic activity using optogenetic and pharmacologic approaches. In transgenic mice specifically expressing halorhodopsin (eNpHR) in cholinergic neurons, photostimulation of striatal ACh interneurons in unilateral 6-OHDA lesioned mice inhibited ACh neuronal activity and reduced the asymetric motor symptoms (postural asymmetry and turning bias). To further investigate the muscarinic cholinergic subtypes involved in these beneficial effects, systemic and intrastriatal injections of telenzepine and tropicamide (M1 and M4 receptor antagonists, respectively) were tested in the same PD model. The beneficial effect on motor symptoms were reproduced by blocking either M1 or M4 mACh receptors in the dorsal striatum. To decipher the mechanisms of ACh action on striatal post-synaptic M4 receptors, additional experiments were performed with mutant mice that lack M4 receptors only in D1 dopamine receptor-expressing cells. As cognitive and neuropsychiatric symptoms are increasingly recognized in PD, we also examined the role of striatal ACh interneurons in these deficits in a bilateral partial model of PD. Blockade of striatal ACh interneurons activity by eNpHR photostimulation or by M1 and M4 receptor antagonists was tested on elevated plus maze, spatial object recognition and social recognition tests. The present results indicate that cholinergic modulation of the dorsal striatal circuit, particularly M1 or M4 receptor subtypes, plays a pivotal role in the regulation of motor, cognitive and emotional symptoms in PD. This work is supported by ANR, France Parkinson, CNRS and AMU, and D1-M4-KO mice were provided by J. Wess (Bethesda, USA)

Disclosures: S. Ztaou: None. **M. Liberge:** None. **N. Maurice:** None. **F. Jouen:** None. **L. Kerkerian-LeGoff:** None. **C. Beurrier:** None. **M. Amalric:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.05/C87

Topic: C.03. Parkinson's Disease

Support: FAPERJ

CNPq

PROPPI-UFF

CAPES

Title: Temporal modulation of striatal cholinergic system and behavior analysis in the 6 hydroxydopamine mouse model of Parkinson`s Disease

Authors: *A. C. FARIA-MELIBEU[1], A. C. M. N. FERNANDES, JR[2], R. C. FIGUEIREDO[2], L. S. HAYASHIDE[2], P. PANDOLFO[2], C. A. SERFATY[2], P. CAMPELLO-COSTA[2], M. G. L. RIBEIRO[2];
[1]Univ. Federal Fluminense, Rio De Janeiro, Brazil; [2]Fluminense Federal Univ., Niterói, Brazil

Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by a progressive and specific degeneration of A9 dopaminergic neurons of the substantia nigra, leading to the classic motor and cognitive deficits. Several studies have shown that cholinergic activity in the striatum regulates the release of dopamine (DA) through the action of nicotinic receptors (nAChRs) which are expressed in dopaminergic nerve terminals. Since the normal activity of striatal neurons depends on the balance between DA and ACh, disruptions in this signaling may contribute to the development of PD. The aim of the present research is to investigate the biochemical changes in the cholinergic system during different survival periods in a mouse model of PD, in order to study the possible striatal cholinergic modulation and the performance of these animals in a memory test. Anaesthetized adult C57Bl6 mice were submitted to unilateral injection of 6-OHDA (2 µl at a rate of 0.5 µl/5 min) into the left medial forebrain bundle using stereotaxic procedures. 1, 2 or 4 weeks after surgery, 6-OHDA and SHAM groups were submitted to the rotational test induced by apomorphine (0.5 mg/kg, ip) and to the object recognition test, to estimate the ability of the animal to distinguish a new object from a familiar one. The amount of time taken to explore the new object provides an index of recognition memory for the investigation of learning processes. Then, the mice were euthanized and striatum used to biochemical assay. Our results showed a modulation of Ach turnover. Moreover, we also detected a reduction in α6 subunit content. Conversely, α7 and β2 subunits content had a progressive increase that was accompanied by improvements in motor behavior. Behavior analyses showed that 6-OHDA mice were not able to distinguish pairs of objects with subtle structural differences. 6-OHDA mice with 2 weeks after lesion surgery showed the worst results in this test, while the animals with 4 weeks showed a partial improvement. Altogether, these data suggest that the availability of ACh may be involved in the remodeling of nAChRs as a result of the dopaminergic loss, what might contribute to partial improvement in a discriminative learning and motor behavior observed in our animals.

Disclosures: **A.C. Faria-Melibeu:** None. **A.C.M.N. Fernandes:** None. **R.C. Figueiredo:** None. **L.S. Hayashide:** None. **P. Pandolfo:** None. **C.A. Serfaty:** None. **P. Campello-costa:** None. **M.G.L. Ribeiro:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.06/C88

Topic: C.03. Parkinson's Disease

Support: FAPESP/2014/22817-1

R01NS089470

Title: Dopamine manipulation disrupts delta/theta activity in medial frontal cortex during cognitive tasks in humans and rodents

Authors: ***R. N. RUGGIERO**[1], K. PARKER[1], Y. KIM[1], J. KINGYON[1], J. CAVANAGH[2], N. NARAYANAN[1];
[1]Dept. of Neurology, Univ. of Iowa, Iowa City, IA; [2]Psychology, Univ. of New Mexico, Albuquerque, NM

Abstract: Abnormal changes in dopamine in the cerebral cortex are characteristic of a series of human neurological disorders such as schizophrenia, ADHD and Parkinson's disease. These conditions are well known to present profound cognitive process alterations. It is still unknown how altered dopamine signaling influences cortical processing. We study this question by manipulating cortical dopamine signaling and studying field potentials in the medial frontal cortex using different approaches. First, we investigated patterns of EEG oscillations during elementary cognitive tasks in humans with Parkinson's. We also used pharmacological tools to block medial frontal dopamine neurotransmission in rodents. Finally, to specifically and selectively manipulate dopamine neurons in the midbrain, we used transgenic mice in which cre-recombinase is expressed only in neurons expressing tyrosine hydroxylase. We injected AAV-LoxP-ChR2 and implanted optical fibers in the VTA, and a recording electrode array in the medial frontal cortex. We record medial frontal neuronal ensembles as we optogenetically change the firing rate of these neurons in rodents performing a fixed interval timing task. Our preliminary results indicate that humans with Parkinson's disease and rodents with disrupted medial frontal dopamine have attenuated 4 Hz oscillations triggered by instructional stimuli during cognitive tasks. We also demonstrate that these oscillations depend on D1 dopamine signaling. These results indicate how dopamine affects media frontal activity influencing cognition. Such findings help to better understand how information processing can be modified in ADHD, schizophrenia and Parkinson's disease.

Disclosures: **R.N. Ruggiero:** None. **K. Parker:** None. **Y. kim:** None. **J. Kingyon:** None. **J. Cavanagh:** None. **N. Narayanan:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.07/C89

Topic: C.03. Parkinson's Disease

Support: NIH Grant 5T32GM067795

Bachmann-Strauss

NIH Grant NS092190

Title: Movement and striatal activity in the development of L-Dopa-induced dyskinesias

Authors: ***S. L. ALBERICO**[1,2], Y. KIM[2], S. J. GROSS[1], N. S. NARAYANAN[2];
[2]Neurol., [1]Univ. of Iowa, Iowa City, IA

Abstract: Parkinson's disease is most commonly treated with the dopamine precursor L-Dopa. Although L-Dopa is effective, a major limitation is that long-term use leads to L-Dopa-induced dyskinesias (LIDs). The mechanism of LIDs remains unknown. Here, we studied the development of LIDs in a mouse model. We injected 6-OHDA into the medial forebrain bundle and implanted microwire electrode arrays in the striatum as well as hardware to track movements. We tested the hypothesis that during LIDs the correlation between movement and striatal neural activity is disrupted. Following dopamine depletion, LIDs were developed with daily injections of L-Dopa (20 mg/kg, i.p.) for 14 days. During the development of LIDs, animal movement was tracked with high temporal and spatial resolution using infrared tracking and accelerometers. Simultaneously, we recorded from neuronal ensembles in the striatum. Previous research demonstrated that the firing rate of neurons in the substantia nigra significantly correlated either positively or negatively with x or y positions. Our striatal data are consistent with this finding. Moreover, our preliminary data suggest that striatal activity and movement correlate less in sessions where LIDs are present. Additionally, animals depleted of dopamine and serotonin exhibit less LIDs. We are currently exploring this phenomenon by pharmacologically inhibiting serotonergic neurons with 8-OH-DPAT, which has been used successfully to decrease LIDs in rats. These data could lead to a deeper understanding of the mechanism of LIDs.

Disclosures: **S.L. Alberico:** None. **Y. Kim:** None. **S.J. Gross:** None. **N.S. Narayanan:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.08/C90

Topic: C.03. Parkinson's Disease

Support: NIH Fellowship 1F31NS080543-03

NIH Grant R01 NS064984

Title: Pathway-specific remodeling of thalamostriatal synapses in Parkinsonian mice

Authors: *P. R. PARKER[1], A. C. KREITZER[2];
[1]Neurosci., UCSF GIND, San Francisco, CA; [2]UCSF/Gladstone Inst., San Francisco, CA

Abstract: Suppression of movement in Parkinson's disease (PD) is thought to arise from increased efficacy of the indirect pathway basal ganglia circuit, relative to the direct pathway. However, the underlying pathophysiological mechanisms remain elusive. To examine whether changes in the strength of synaptic inputs to these circuits contribute to this imbalance, we obtained paired whole-cell recordings from striatal direct- and indirect-pathway medium spiny neurons (dMSNs and iMSNs) and optically stimulated inputs from sensorimotor cortex or intralaminar thalamus in brain slices from control and dopamine-depleted mice. We found that dopamine depletion selectively decreased synaptic strength at thalamic inputs to dMSNs, suggesting that thalamus drives asymmetric activation of basal ganglia circuitry underlying parkinsonian motor impairments. Consistent with this hypothesis, *in vivo* chemogenetic inhibition of intralaminar thalamic neurons reversed motor deficits in dopamine-depleted mice. These results implicate thalamostriatal projections in the pathophysiology of PD and support interventions targeting thalamus as a potential therapeutic strategy.

Disclosures: P.R. Parker: None. **A.C. Kreitzer:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.09/C91

Topic: C.03. Parkinson's Disease

Title: Electrophysiological study of the substantia nigra reticulata activity in a rat model of l-dopa induced dyskinesia

Authors: *L. UGEDO[1], A. ARISTIETA[2], C. MIGUELEZ[2], T. MORERA_HERRERAS[2], J. A. RUIZ-ORTEGA[3];
[2]Pharmacology, Med. Sch., [1]Univ. Basque Country, Bizkaia, Spain; [3]Pharmacology, Pharm. Sch., Univ. Basque Country, Araba, Spain

Abstract: The pathophysiology of Parkinson's disease (PD) and L-DOPA induced dyskinesias (LID) is associated with dysfunctional neuronal activity in several nuclei of the basal ganglia. Also high level of oscillatory activity and synchronization have been described, both intra and inter the basal ganglia nuclei and the cerebral cortex. However, the relevance of these alterations in the motor symptomatology associated to two different stages of parkinsonism is not fully understood. Recently, we have shown that the subthalamic neuronal activity correlates with axial dyskinetic movements and that subthalamic nucleus (STN) lesion partially reduces dyskinesia severity as well as the expression of some striatal molecular modifications (Aristieta et al., 2012, Plos one 7; e42652-e42652). In the present study we further investigate the electrophysiological changes and the role of the STN in parkinsonism and LID. Neuronal activity of the substantia nigra reticulata (SNr), the STN and cerebral cortex were recorded from 6-OHDA lesioned and dyskinetic rats. Results show that the firing rate of SNr neurons from dyskinetic animals was increased with respect to values obtained in intact and 6-OHDA lesioned rats. Moreover, there was a significant correlation ($p<0.01$) between the mean firing rate of SNr neurons and the severity of the dyskinetic movements (limb and orolingual subtypes). We also found a significant correlation between the firing activity of SNr and STN neurons recorded from dyskinetic rats. In addition, 6-OHDA lesioned animals showed low frequency band oscillatory activity and synchronization both, within the nucleus and with the cerebral cortex, regardless the chronic treatment with L-DOPA. The degree of synchronization and oscillatory activity in parkinsonian and dyskinetic rats was higher with respect Altogether, these results indicate that SNr neuronal firing activity is relevant in dyskinesia and may be driven by STN hyperactivity. On the other hand, oscillatory activity and synchronization seem to be more important in PD since they are not influenced by prolonged L-DOPA administration. Supported by GIC IT747-13. There is no conflict of interest.

Disclosures: **L. Ugedo:** None. **A. Aristieta:** None. **C. Miguelez:** None. **T. Morera_Herreras:** None. **J.A. Ruiz-Ortega:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.10/C92

Topic: C.03. Parkinson's Disease

Support: NIH grant 1DP2NS082126

NINDS 1R01NS087950

NINDS 1R21NS078660

NINDS 1R01S081716

NIMH 5R00MH085944

PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: Muscarinic dependent cholinergic modulation of striatal beta oscillations

Authors: *K. KONDABOLU[1], E. A. ROBERTS[2], M. BUCKLIN[2], M. M. MCCARTHY[2], N. KOPELL[2], X. HAN[2];
[1]Boston Univ., Malden, MA; [2]Boston Univ., Boston, MA

Abstract: Parkinson's disease is characterized by degeneration of mid-brain dopaminergic neurons that project to the striatum. In animal models of dopamine depletion, striatal cholinergic tone is elevated and is coincident with enhanced pathological beta oscillations (15-30Hz) within the cortico-basal ganglia-thalamic circuit. Recently, we have demonstrated that infusion of cholinergic agonist in the striatum generates exaggerated beta oscillations - consistent with our computational model - suggesting that striatal circuitry possessed the ability to generate beta oscillations. In the current study, we utilized optogenetics to directly activate striatal cholinergic interneurons, and demonstrate that increased cholinergic tone is sufficient to produce beta oscillations within the striatum. We also show that these beta oscillations can propagate to the motor cortex in a cortical layer dependent manner, with the deeper layers having the greatest increase in beta power. Furthermore, we demonstrate that the muscarinic receptors, but not the nicotinic receptors are critical for generation of beta oscillations. Together, our results provide direct evidence for striatal cholinergic interneurons ability to generate propagating beta oscillations through activating muscarinic receptors.

Disclosures: K. Kondabolu: None. **E.A. Roberts:** None. **M. Bucklin:** None. **M.M. Mccarthy:** None. **N. Kopell:** None. **X. Han:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.11/C93

Topic: C.03. Parkinson's Disease

Title: High striatal cholinergic tone attenuates theta-gamma coupling in M1 and striatum

Authors: *M. ROMANO[1], B. PITTMAN-POLLETTA[2], M. MCCARTHY[2], K. KONDABOLU[3], N. KOPELL[2], X. HAN[3];
[1]Grad. Program in Neurosci., [2]Dept. of Mathematics & Statistics, [3]Dept. of Biomed. Engin., Boston Univ., Boston, MA

Abstract: Locally synchronous neuronal firing generates cognitive rhythms, some of which have been associated with movement disorders. Rhythms might not only serve as means of communication between different brain regions, but might also influence rhythms of other frequencies. In a recent study, theta (4-8 Hz) rhythms were shown to phase modulate gamma (30-100 Hz) both within the motor cortex (M1) and within the striatum while mice were running (von Nicolai 2014). This suggests that theta-gamma coupling within M1 and within striatum may have roles in movement. Here we show that increased cholinergic tone in striatum decreases this phase-amplitude coupling in both of these regions. We simultaneously recorded local field potentials from M1 and dorsolatereral striatum in Chat-ChR2 mice, with channelrhodopsin expressed in striatal cholinergic interneurons. We used wavelets to extract amplitudes and phases, and used a modulation index (MI) to measure cross-frequency coupling between low bands of 0.5-30 Hz and phase-modulated bands of 8-200 Hz. We find a decrease in coupling compared to non-stimulation time periods between low theta (~4 Hz) and high gamma (~75 Hz) in M1 with stimulation of the striatal cholinergic interneurons. A similar trend of phase-amplitude coupling was also seen in striatum. However, we also found an increase over control in theta-gamma coupling in both M1 and striatum during the time periods between stimulation. Such an increase may be a response to rebound of dopamine in striatum (López-Azcárate 2013). These results have importance as the state of striatal cholinergic tone is relevant to Parkinson's disease, a disease in which bradykinesia (slowness of movements) is a characteristic symptom. Increases in beta and decreases in gamma are characteristic oscillatory changes seen in the Parkinsonian state, which can be produced by increasing cholinergic tone in striatum (McCarthy 2011). References 1. López-Azcárate J, Nicolas MJ, Cordon I, Alegre M, Valencia M, and Artieda J (2013) Delta-mediated cross-frequency coupling organizes oscillatory activity across the rat cortico-basal ganglia network. Frontiers in Neural Circuits 7(155):1-16. 2. McCarthy MM, Moore-Kocklacs C, Gu X, Boyden ES, Han X, and Kopell N (2011) Striatal origin of the pathologic beta oscillations in Parkinson's Disease. PNAS 108(28):11620-11625. 3. von Nicolai C, Engler G, Charott A, Engel AK, Moll CK, and Siegel M (2014) Corticostriatal Coordination through Coherent Phase-Amplitude Coupling. The Journal of Neuroscience 34(17):5938-5948.

Disclosures: M. Romano: None. **B. Pittman-Polletta:** None. **M. McCarthy:** None. **K. Kondabolu:** None. **N. Kopell:** None. **X. Han:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.12/C94

Topic: C.03. Parkinson's Disease

Support: NIH grant 1DP2NS082126

NINDS 1R01NS087950

NINDS 1R21NS078660

NINDS 1R01S081716

NIMH 5R00MH085944

PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: Striatal cholinergic and dopaminergic tone modulate distinct beta band sub-frequencies in the cortical-basal ganglia-thalamic loop

Authors: *A. QUACH, B. R. PITTMAN-POLLETTA, N. KOPELL, M. MCCARTHY, X. HAN;
Boston Univ., Boston, MA

Abstract: Enhanced oscillations in the beta frequency band (8-30 Hz) are a characteristic neural signature observed in the cortical-basal ganglia-thalamic (CBT) loop of Parkinson's disease (PD) patients. While beta oscillations have been shown to be correlated with parkinsonian motor symptoms, the mechanisms underlying the generation and propagation of beta oscillations in PD are not well-understood. We have previously shown both computationally and experimentally that increased cholinergic activation in the striatum can lead to enhanced beta oscillations in normal, non-parkinsonian mice (McCarthy et al, 2011). Here, we infused the acetylcholine agonist carbachol into the striatum of normal adult mice to produce beta oscillations and recorded simultaneously from the striatum and the subthalamic nucleus (STN) or the motor cortex (M1). We found that striatal carbachol-induced beta oscillations can propagate to STN and M1. However, STN and M1 manifest distinct oscillation patterns at different sub-frequencies within the beta band. Broadband beta oscillations increased in STN, but only the highest beta band sub-frequency (18-25 Hz) in M1 increased. We then optogenetically silenced excitatory neurons in deep layers of M1, and observed a reduction only in low beta (8-13 Hz) in M1 but no change in the striatum, suggesting that in the exaggerated cholinergic state, striatal beta oscillations are independent of cortical input and that only low beta in M1 is dependent on the activity of cortical excitatory neurons. Finally, we compared these results to recordings from M1 and striatum in a 6-hydroxydopamine (6OHDA) model of PD. The exact same pattern of beta sub-band elevation was observed in the 6OHDA mice as in the carbachol-infused mice with the exception of the low beta band in M1, which was elevated with 6OHDA but not with carbachol. Additionally, optogenetic silencing of M1 excitatory neurons paradoxically increased low beta in M1 in the 6OHDA mice and decreased all sub-bands of beta in striatum. Together, our results provide direct evidence that cholinergic and dopaminergic tone differentially modulate

propagating beta oscillations within the CBT loop. At least two beta oscillators were observed in the motor cortex with the low sub-frequency beta oscillator strongly dependent upon M1 local circuitry whereas the high sub-frequency beta oscillator strongly modulated by basal ganglia circuits. Dopamine depletion and cholinergic upregulation created similar patterns of striatal beta oscillation patterns, but differentially impacted striatal responses to M1 inputs.

Disclosures: A. Quach: None. **B.R. Pittman-Polletta:** None. **N. Kopell:** None. **M. McCarthy:** None. **X. Han:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.13/C95

Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS46058

NSF DMS-1042134 to NK

Title: A possible mechanistic link between beta oscillations and bursting in the Parkinsonian basal ganglia

Authors: *M. M. MCCARTHY, B. R. PITTMAN-POLLETTA, A. QUACH, X. HAN, N. KOPELL;
Boston Univ., Boston, MA

Abstract: Increased neuronal busting and increased oscillatory activity in the beta frequency (8 - 30 Hz) range are two prominent electrophysiological characteristics of the parkinsonian basal ganglia. It is currently unknown if there is a mechanistic link between them. Here we use a combination of experimental and computational techniques to investigate the possibility of a direct association between bursting and beta oscillations in basal ganglia. We have previously shown that increased striatal cholinergic tone, a condition relevant to Parkinson’s disease, reliably and reversibly produces exaggerated beta oscillations in the basal ganglia of normal mice. Here we find that high striatal cholinergic tone not only increases the power of beta oscillations but also changes the phase relationships in the beta frequency range between basal ganglia nuclei, specifically between the striatum and the subthalamic nucleus (STN). Striatal beta oscillations generally phase lead STN beta oscillations under conditions of normal striatal cholinergic tone. In contrast, under conditions of high striatal cholinergic tone, STN phase leads striatum over a wide region of the beta frequency band (13 - 23 Hz). We use computational models to examine the effect of this beta frequency phase shift on the output nucleus of the basal ganglia, the internal segment of the globus pallidus (GPi), which receives input from both striatum and STN. We find significantly increased bursting in GPi when STN input precedes

striatal input. This is due to a relatively high rate of coincident spiking between GPi and STN and thus increased activation of NMDA-mediated currents in GPi. In contrast, when striatal input precedes STN input, GPi neurons are hyperpolarized during the time of arrival of STN input, which effectively prevents NMDA-induced bursting in GPi. These results suggest a potential mechanistic link between beta oscillations and neuronal bursting in GPi under conditions of high striatal cholinergic tone. They further suggest the beta phase difference between the striatum and the STN, as opposed to the power of the beta oscillations, may be more immediately linked to the dysfunction associated with the beta oscillations in the parkinsonian basal ganglia.

Disclosures: **M.M. McCarthy:** A. Employment/Salary (full or part-time):; Boston University. **B.R. Pittman-Polletta:** None. **A. Quach:** None. **X. Han:** None. **N. Kopell:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.14/C96

Topic: C.03. Parkinson’s Disease

Support: NIH Grant 1DP2NS082126

NINDS 1R01NS087950

NINDS 1R21NS078660

NINDS 1R01S081716

NIMH 5R00MH085944

PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: Enhanced cortico-striatal beta oscillations and synchrony mediated by optogenetic increase of striatal cholinergic tone

Authors: *E. A. ROBERTS, K. KONDABOLU, M. ABDULKERIM, M. M. MCCARTHY, N. KOPELL, X. HAN;
Boston Univ., Boston, MA

Abstract: Amplified beta frequency (13-30Hz) oscillations in the cortico-basal ganglia-thalamic (CBT) loop are characteristic of Parkinson’s disease (PD). Enhanced beta spectral power is known to correlate with bradykinesia and akinesia symptoms in PD patients. Another feature of PD is increased beta synchrony between the basal ganglia and cerebral cortex. Clinically, dopamine replacement and basal ganglia deep brain stimulation attenuate beta power and

cortical-basal ganglia synchrony, leading to improvements in bradykinesia and akinesia. Since anticholinergic medication has also been used in humans to reduce PD tremor, it is not surprising that animal models of Parkinson's disease demonstrate augmented striatal cholinergic tone. Previous studies from our group demonstrated that injection of a cholinergic agonist into striatum leads to increased beta power, suggesting that striatum can generate beta oscillations. However, it was undetermined if these cholingerically mediated beta oscillations propagated through the CBT loop. To investigate this, we optogenetically activated striatal cholinergic interneurons in mice while simultaneous recording from the striatum as well as the motor cortex using a laminar probe. Optogenetic increase in cholinergic tone produced beta oscillations in the striatum that propagated to motor cortex in a cortical depth dependent manner. This was accompanied by a simultaneous increase in beta coherence between these structures. Behavioral activity of mice was monitored in a separate activity chamber to confirm that these beta oscillations affected locomotion. We observed a laser induced decrease in locomotion that was restored back to baseline activity after laser offset. These results demonstrate that optogenetically increasing striatal cholinergic tone, a state relevant to PD, produces pathophysiological oscillations and behavior typical of PD.

Disclosures: E.A. Roberts: None. **K. Kondabolu:** None. **M. Abdulkerim:** None. **M.M. McCarthy:** None. **N. Kopell:** None. **X. Han:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.15/D1

Topic: C.03. Parkinson's Disease

Title: Spiking neuron model of the basal ganglia for the generation of Parkinsonian pathological oscillations

Authors: *O. SHOUNO[1,2], K. DOYA[2];
[1]Honda Res. Inst. Japan Co Ltd, Saitama, Japan; [2]Okinawa Inst. of Sci. and Technol. Grad. Univ., Tancha, Onna-son, Okinawa, Japan

Abstract: Parkinson's disease is a disorder of movement caused by dopamine depletion in the basal ganglia. Abnormally synchronized neuronal oscillations between 8 Hz and 15 Hz in the basal ganglia are implicated in motor symptoms of Parkinson's disease. However, how these abnormal oscillations are generated and maintained still remains unveiled. Based on recent experimental evidence (Atherton et al., 2013), we propose a hypothesis that the short-term plasticity of the synapses between the subthalamic nucleus (STN) and the external segment of the globus pallidus (GPe), as well as post-inhibitory rebound bursting of STN neurons, generate parkinsonian oscillations and that the cortical oscillatory feedback input to the STN can amplify

the oscillation. To test this hypothesis, we develop a spiking neuron model of the STN-GPe circuit and performed systematic search in the model parameter space to reproduce BG neuronal activities in normal and parkinsonian states (Tachibana et al., 2011). Simulation results revealed that the mean firing rates, oscillation frequency, and the enhanced bursting rate of parkinsonian oscillation are reproduced by reduced excitability of GPe neurons and increased strength of GPe-to-STN synaptic connections. The reduced activity of GPe neurons can be caused by reduced autonomous activity of GPe neurons (Chan et al., 2011) or increased inhibitory input from striatal D2R expressing neurons under dopamine depletion. The increased GPe-to-STN inhibitory connection, combined with its short-term depression property, triggers stronger rebound burst activities of STN neurons. Furthermore, oscillatory cortical feedback input to the STN amplifies 8-15 Hz oscillation if the feedback delay of the GPi-thalamus-cortex-STN loop is within 60-80 ms, while increased tonic excitatory cortical input to the STN can suppress the oscillations. These observations will be the basis for identifying new targets for the therapy of Parkinson's disease, such as deep brain stimulation.

Disclosures: **O. Shouno:** None. **K. Doya:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.16/D2

Topic: C.03. Parkinson's Disease

Support: Medical Research Council

Title: Phase coupling between neural populations in Parkinson's disease

Authors: ***H. CAGNAN**[1,2], A. SHAROTT[2], N. MALLET[3], P. MAGILL[2], P. BROWN[2];
[1]Univ. Col. of London, London, United Kingdom; [2]Univ. of Oxford, Oxford, United Kingdom; [3]Univ. of Bordeaux, Bordeaux, France

Abstract: Phase coupling between neural populations is believed to shape information flow and determine system performance. In Parkinson's disease (PD) the motor circuit exhibits excessively synchronized rhythmic activity patterns. These activity patterns are correlated with PD motor symptoms such as bradykinesia, rigidity and tremor, suggesting that excessive synchrony could underlie impaired motor system performance. Using simultaneously recorded local field potential (LFP) recordings from the subthalamic nucleus (STN) and the globus pallidus (GP), we investigated the neural processes underlying excessive synchronisation and its termination in PD [Cagnan et. al. Brain, 2015 (doi: 10.1093/brain/awv093)]. Local beta activity in each nucleus varied in amplitude according to the phase alignment between the nuclei, and according to how long such phase alignment was sustained. LFPs were more likely to align at

phases that amplified local beta synchrony when patients were off as opposed to on dopaminergic therapy, and did so for longer, thereby promoting beta activity. Dopaminergic stimulation shifted the motor circuit from prolonged periods of phase locking enhancing beta synchrony, associated with PD motor symptoms, to more dynamic phase coupling and improved motoric function. Here we corroborate the above findings in the 6-OHDA lesioned rodent model of PD, extending them to multi-unit activity and establishing the frequency specificity of phase alignment effects. Critically, by focusing on multiunit activity rather than LFP activity, we have the spatial resolution to establish that local amplitude amplification also entails progressively more spatially extensive intranuclear synchronization. Together our findings demonstrate the dynamic interaction between internuclear phase alignment and intranuclear synchronization, and its control by the neuromodulator dopamine in the basal ganglia circuit.

Disclosures: H. Cagnan: None. **A. Sharott:** None. **N. Mallet:** None. **P. Magill:** None. **P. Brown:** None.

Poster

302. Circuit Mechanisms in Parkinson's Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 302.17/D3

Topic: C.03. Parkinson’s Disease

Title: Gamma band favors levodopa-induced dyskinesia: an *in vivo* study on freely-moving rats

Authors: V. D'ANGELO[1], *A. STEFANI[1], A. SALVADÉ[2], A. KAELIN[2,3], S. GALATI[2]; [1]Univ. Tor Vergata, Rome, Italy; [2]Neurocenter of Southern Switzerland, Torricella-Taverne, Switzerland; [3]Movement Disorders Ctr., Bern, Switzerland

Abstract: An enhanced β band (βB) oscillatory neuronal activity features basal ganglia discharge in Parkinson’s disease (PD), as evaluated during stereotactic neurosurgery in humans as well as in disease rodent’s models. Far from fully elucidated is whether higher βB partecipates to the behavioral pathogenesis or represents a mere correlative hallmark. A less explored phenomenon regards the putative contribution of the γ band (γB) activity to PD pathophysiology. We have monitored these frequency bands thorugh weekly histological and electrophysiological assessments (from unilateral globus pallidus - GP - and bilateral frontal cortex) in freely moving rats, following traditional unilateral 6-HPDA lesioning; hence, we acquired parameters right after denervation, after 2-4-6 weeks, and after levodopa therapy (in groups exhibiting or not severe involuntary movements). It was detected a significant increase of the βB (18-30 Hz) activity within the first week after the toxin injection, while γB remained quite constant. Notably, the increase βB discharge affected also cortex contralateral to the lesion. Dopaminergic treatment influenced in opposite ways the two examined bands, reverting βB but increasing the expression of the γB in those animals manifesting dyskinesia. If, on one hand, βB activity increased parallel

to the emerging of PD akinesia and impaired TH-staining, on the other hand the occurrence of dyskinesia correlated strongly with γB activity. We are suggesting that, albeit a *normal* γB exerts a physiological pro-kinetic role, an exhuberant cortical γB influences the unmasking and intensity of dyskinesia.

Disclosures: V. D'Angelo: None. **A. Stefani:** None. **A. Salvadé:** None. **A. Kaelin:** None. **S. Galati:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.01/D4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Office of Research and Sponsored Programs at Central Michigan University

College of Medicine at Central Michigan University

John G. Kulhavi Professorship

The Field Neurosciences Institute

Dr. Mary P. Dole Medical Fellowship

Title: Neuronal, glial and neural stem cell marker expression in mesenchymal stem cells from R6/2 (transgenic mouse model of Huntington's disease)

Authors: *C. B. KAYA[1], M. LU[2], K. L. RETERSTORF[3], T. S. WHITE[4], G. DUNBAR[2], J. ROSSIGNOL[5];
[2]Psychology, [3]Biochem., [4]Engin. and Technol., [5]Col. of Med., [1]Central Michigan Univ., Mount Pleasant, MI

Abstract: Current research data on the use of stem cells as cell replacement therapy for neurological diseases show inconsistent results on whether mesenchymal stem cells (MSC) are capable of neuronal, glial and/or neural progenitor differentiation during subsequent *in vitro* culturing in the absence of complex induction media. Bone marrow-derived MSCs isolated from R6/2 transgenic mouse model for Huntington's disease and its wild-type counterpart, were isolated, cultured and analyzed by flow cytometry, immunocytochemistry and qRT-PCR. Preliminary data show varying, positive expression of neuronal, glial, neural progenitor and basal MSC markers. Such discrepancies in MSC, glial, neural progenitor and neuronal biomarker expression across passages, and between wild-type and Huntington's Disease-positive MSCs implies feasibility of isolating a putative multipotent adult progenitor (MAP-MSC) cell population in MSCs that are predisposed to neuronal, glial and/or neural progenitor

differentiation. Naïve MSCs are therefore, found to be 'differentiation-ready'. Selecting for such subpopulations of MSCs, directed to have a particular neural lineage without the need for any induction or differentiation media, may enhance stem cell transplantation studies for neurodegenerative diseases and also allow for a faster turnaround from isolation to transplantation, and thus offering better transplantation survivability.

Disclosures: C.B. Kaya: None. **M. Lu:** None. **K.L. Reterstorf:** None. **T.S. White:** None. **G. Dunbar:** None. **J. Rossignol:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.02/D5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant 5 RO1 NS038194-15

Title: Huntingtin mRNA 3′UTR isoform abundance changes in a mouse model of Huntington's disease

Authors: *L. ROMO[1], E. PFISTER[1], A. ASHAR-PATEL[2], M. MOORE[2], N. ARONIN[1]; [1]Med., [2]RNA Therapeut. Inst., Umass Med. Sch., Worcester, MA

Abstract: Huntington's disease is a devastating neurodegenerative disorder caused by an expanded glutamine repeat within exon 1 of the huntingtin (HTT) gene. The wild-type and mutant allele are expressed as two alternatively polyadenylated (APA) mRNA isoforms with tissue-specific abundance. Isoforms differ only in the length of their 3′ untranslated region (3′UTR), and both are processed into the HTT protein. Recent findings indicate mutant and wild-type mRNA exhibit different localization and stability. These differences may be due to alternative processing of mutant and wild-type mRNA into different isoforms. Here, we aimed to determine if the abundance of HTT 3′UTR isoforms changes in a model mouse. Quantitative PCR assessed the abundance of the HTT long 3′UTR isoform mRNA in male Yac18-/- and Yac128-/- mice after normalization to total HTT. These mice lack endogenous HTT but harbor the full-length human HTT gene with either expanded 128 (Yac128) or wild-type 18 (Yac18) glutamine repeats. We found Yac128 mouse tissues contain a significantly higher abundance of long HTT isoform than Yac18 mouse tissues. To determine if this change was specific to HTT, we performed next generation polyadenylation (polyA) site sequencing on Yac18 or Yac128 mouse cortex. Of 1,466 genes with two detected 3'UTR polyA sites, none exhibit significantly ($p<0.01$) different isoform ratios between Yac128 and Yac18 cortex. Interestingly, despite reported transcriptional dysregulation in symptomatic disease, only 82 mRNAs out of 11,469 show significant changes in polyA mRNA expression. The difference in total HTT expression

between Yac128 and Yac18 mice was less than the difference in HTT long isoform expression, implying the shift to the long HTT isoform in Yac128 mice is not solely explained by transcriptional changes. These data indicate disease-associated changes in HTT isoform abundance are unique to HTT, suggesting they are due to a cis element rather than a change in an APA global regulator. It remains unclear what HTT cis element dictates the observed changes in 3′UTR mRNA isoform abundance, although the promoter-exon1 region is a likely candidate given its role in polyA factor assembly and site selection as well as its disruptive glutamine repeat. These studies reveal differences in HTT mRNA isoform abundance in mouse models of HTT. Our preliminary data suggests these differences also occur in human disease brain. If so, differences in HTT mRNA 3′UTR isoform abundance could explain different stability and localization of the mutant and wild-type allele, and the long 3′UTR will be the ideal target for small RNAs aiming to degrade mutant but not wild-type HTT mRNA.

Disclosures: L. Romo: None. **E. Pfister:** None. **A. Ashar-Patel:** None. **M. Moore:** None. **N. Aronin:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.03/D6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: A*STAR

NUS

Title: Assessment of demyelination/remyelination responses in cuprizone-treated YAC128 Huntington’s disease mice

Authors: *R. TEO[1], C. FERRARI BARDILLE[1], A. TAN[1], M. R. HAYDEN[1,2,3], M. A. POULADI[1,3];
[1]TLGM, Singapore, Singapore; [2]Ctr. for Mol. Med. and Therapeut., Vancouver, BC, Canada; [3]Dept. of Med., Yong Loo Lin Sch. of Medicine, Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Huntington disease (HD) is an autosomal dominant neurodegenerative disorder characterized by motor, cognitive, and psychiatric disturbances. While generally considered a grey matter disease, early and progressive white matter (WM) atrophy is a well-recognized feature of HD. Abnormalities in WM and myelination have also been observed in a number of animal models of HD. Despite this, the aetiology of WM pathology in HD remains largely unexplored. The capacity to generate new oligodendrocytes and new myelin under normal conditions and in response to injury are essential to maintain WM health and function. Whether these processes are impaired in HD has not been addressed. In this study we employed

cuprizone-induced demyelination to characterize the demyelination and remyelination responses in the transgenic YAC128 mouse model of HD. The YAC128 HD mice exhibit phenotypes that mimic many features of human HD including progressive motor, cognitive, affective deficits as well as selective striatal atrophy and white matter abnormalities. To induce demyelination, YAC128 mice and littermate controls were treated with 0.2% cuprizone for 6 weeks starting at 2 months of age. To allow remyelination, cuprizone treatment was withdrawn and the animals were allowed to recover for 6 weeks. Assessment of oligodendroglial (CAII-, GSTpi-, and NG2-positive), microglial (Iba-1-positive), and astrocytic (GFAP-positive) cell counts indicates altered demyelination/remyelination responses in the YAC128 HD mice compared to littermate controls. Gross and ultra-structural myelination characteristics are being examined using myelin basic protein staining and electron microscopy analysis, respectively. Taken together, these studies shed light on remyelination capacity, a process important in the maintenance of white matter integrity, in the HD context.

Disclosures: **R. Teo:** None. **C. Ferrari Bardille:** None. **A. Tan:** None. **M.R. Hayden:** None. **M.A. Pouladi:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.04/D7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: University of Lausanne, Department of Physiology

Title: Neurons and astrocytes contributions to striatal degeneration and behavioral deficits in Huntington’s disease

Authors: *C. MEUNIER[1], N. MERIENNE[2], C. JOLLE[1], M. REY[2], C. PYTHOUD[2], D. NICOLE[2], L. PELLERIN[1];
[1]Dept. of physiology, UNIL, Lausanne, Switzerland; [2]Lab. of Cell. and Mol. Neurotherapies (LCMN), Dept. of Clin. Neurosci., Lausanne Univ. Hosp. (CHUV), Lausanne, Switzerland

Abstract: Huntington’s disease (HD) is a rare neurodegenerative disease caused by an autosomal dominant mutation on the huntingtin gene (*HTT*). Despite ubiquitous expression of the mutant *HTT,* a selective vulnerability of medium-sized spiny neurons (MSN) of the striatum is observed. Recent data have suggested the implication of non-neuronal cells in the disease, in particular astrocytes, which have essential roles in many neuronal functions including ion regulation or energetic modulation. These observations underline the need to better characterize neuron-astrocyte interactions specifically in HD. Several transgenic mouse models of HD are available but modulation of mutant *HTT* expression to reach a cell-type specific pathology is

hardly achieved due to their variability in terms of genetic background and transgene constructions. An alternative strategy consists to model the disease using cell-type specific viral vectors expressing mutant *HTT* in the striatum. In particular, Adeno-Associated Viruses (AAV) offer the possibility to shift their tropism from neurons to astrocytes using specific capsids and cell-type specific promoters. In this study, we characterized several viral-mediated HD models using an AAV2/5 to express a short mutant *HTT* fragment under the control of a neuronal or an astrocytic promoter. We first show that the combination of AAV2/5 and the chicken β actin (CBA) promoter or Gfa2(b)3 promoter leads to a high expression of a reporter gene specifically in striatal neurons or in astrocytes, respectively. We replaced this reporter gene by a cassette expressing the first 171aa of the *HTT* with 82 or 18 CAG to study the contribution of each cell population to HD. Expression of mutant HTT selectively in neurons leads to progressive motor alterations and increased anxiety, whereas expression in astrocytes leads to a less severe phenotype. We furthermore characterized several cellular and molecular hallmarks and their evolution in these two models. Two profiles of huntingtin aggregation were observed between the neuronal and the astrocytic model, leading to specific cellular dysfunctions. We are currently using co-injections to express mutant *HTT* both in neurons and in astrocytes to evaluate the potential synergistic effect of the mutant protein in both cell types. In this study, we were able to make a direct comparison between neuron-specific, astrocyte-specific or more global expression of mutant *HTT* to better understand neuron-astrocyte interactions, and their respective contribution to behavioral and cellular alterations in HD.

Disclosures: **C. Meunier:** None. **N. Merienne:** None. **C. Jolle:** None. **M. Rey:** None. **C. Pythoud:** None. **D. Nicole:** None. **L. Pellerin:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.05/D8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI

Title: Neurophysiological correlates of motivation and timing in a mouse model of Huntington’s disease

Authors: ***N. E. ZLEBNIK**, E. A. COLE, I. GILDISH, D. P. COVEY, J. F. CHEER;
Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Huntington’s disease (HD) is an inherited neurodegenerative disorder caused by a polyglutamine expansion in the huntingtin gene and results in the progressive loss of striatal and cortical neuropil. Striatal neurodegeneration occurs first and mainly affects the indirect pathway

of the basal ganglia, causing dyskinesia followed later by akinesia. However, the marked motor impairments characteristic of HD may often be preceded by motivational and cognitive dysfunction. Recent studies report altered function at dopamine terminals in in HD patients and rodent models, but how this relates to motivational and cognitive deficits is unclear. In order to characterize mesolimbic system dynamics in HD progression, extracellular electrophysiological recordings were collected at multi-electrode arrays implanted in the nucleus accumbens of wild-type (WT) and Q175 knock-in mice during tasks of motivation (progressive ratio) and timing (fixed-interval 30). While reward-encoding single units exhibited similar patterns in both genotypes, session-wide power distribution significantly differed across several frequency bandwidths and in reward-evoked power specifically in the gamma bandwidth. These results suggest the nucleus accumbens may be one brain locus with sufficient predictive power to function as a neurophysiological biomarker of the motivational and cognitive impairments inherent to HD.

Disclosures: **N.E. Zlebnik:** None. **E.A. Cole:** None. **I. Gildish:** None. **D.P. Covey:** None. **J.F. Cheer:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.06/D9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Swiss National Science Foundation 31003A-140945

Title: Genetic editing with the CRISPR/Cas9 system for Huntington’s disease

Authors: ***N. MERIENNE**[1], C. MEUNIER[2], V. ZIMMER[1], G. PERRIARD[3], M. CANALES[3], G. VACHEY[1], L. HERRGOTT[1], T. BELTRAMINELLI[1], T. DEQUESNE[1], C. PYTHOUD[1], M. REY[1], R. DU PASQUIER[3], A. L. PERRIER[4], L. PELLERIN[2], N. DÉGLON[1];
[1]CHUV, Lausanne, Switzerland; [2]Dept. of Physiology, Lab. of neuroenergetics, Univ. of Lausanne, Lausanne, Switzerland; [3]Lab. of Neuro-immunology, Dept. of Clin. Neurosciences (DNC), Lausanne Univ. Hosp. (CHUV), Lausanne, Switzerland; [4]UEVE U861, I-STEM, AFM, Inst. Natl. de la Santé et de la Recherche Médicale (INSERM), Evry, France

Abstract: Huntington’s disease (HD) is a neurodegenerative disorder caused by a pathological CAG expansion at the 3’ end of the first exon of the huntingtin gene (*HTT*). Currently, there is no efficient treatment for HD. Editing of the mutant *HTT* gene with the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system represents a new and promising approach. Recognition of the *HTT* target sequence by a single-guide RNA sequences (sgRNA) and the Cas9 protein is inducing DNA double-strand breaks (DSB), which activate endogenous

cellular repair pathways. Non-homologous end joining (NHEJ) will introduce small insertions/deletions (indels) that alter the reading frame of *HTT* gene while homologous directed repair (HDR) is activated in the presence of a DNA template. Both approaches would lead to a definitive loss of mutant *HTT* expression. To validate the approach and optimize the delivery of the CRISPR system with viral vectors, we first targeted artificial sequences containing fluorescent reporter genes in HEK 293T cells. Furthermore, targeting of a genomic integrated reporter gene in neurons and in astrocytes resulted in an efficient gene disruption and was associated with a loss of fluorescence *in vitro* and in the mouse striatum. We developed multiple strategies to disrupt the mutant *HTT* gene. Quantification demonstrated a high rate of indels, leading to a strong reduction of HTT protein in HEK 293T cells, mouse cortical neurons and human iPSC-derived neurons. Blocking *HTT* expression in cellular HD models improved several physiopathological parameters. We are currently evaluating the impact of allele or non-allele specific mutant *HTT* editing in human neurons from HD patients. Altogether, these data demonstrate the potential of the CRISPR technology as therapeutic strategy for HD.

Disclosures: **N. Merienne:** None. **C. Meunier:** None. **V. Zimmer:** None. **G. Perriard:** None. **M. Canales:** None. **G. Vachey:** None. **L. Herrgott:** None. **T. Beltraminelli:** None. **T. Dequesne:** None. **C. Pythoud:** None. **M. Rey:** None. **R. du Pasquier:** None. **A.L. Perrier:** None. **L. Pellerin:** None. **N. Déglon:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.07/D10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CNPq Grant 467220/2014-0

CNPq Grant 306840/2014-6

FAPEMIG Grant 00271-13

Title: Changes in structure and function of diaphragm neuromuscular junctions from BACHD mouse model for Huntington’s disease

Authors: *C. GUATIMOSIM[1], B. ARAGAO[1], H. RODRIGUES[1], P. VALADAO[1], W. CAMARGO[2], L. NAVES[2], F. RIBEIRO[3];
[1]Univ. Federal De Minas Gerais, ICB, Dept. De Morfologia, Belo Horizonte, Brazil; [2]Univ. Federal de Minas Gerais, ICB, Dept. de Fisiologia e Biofisica, Belo Horizonte, Brazil; [3]Univ. Fedearl de Minas Gerais, ICB, Dept. de Bioquimica e Imunologia, Belo Horizonte, Brazil

Abstract: Huntington's disease (HD) is a neurodegenerative disorder characterized by a progressive decline of motor and cognitive functions. It is caused by a polyglutamine expansion in the huntingtin (htt) protein which then leads to neuron degeneration involving multiple neuronal compartments that span both the central and peripheral nervous system. At the synaptic level, htt binds to synaptic vesicles and interacts with several proteins associated with vesicular transport. Therefore, it is suggested that the mutant htt may interfere with the release of neurotransmitters causing synaptic dysfunction. In this work, we looked at alterations in diaphragm muscle neuromuscular junctions (NMJs) from BACHD mouse model for HD. This mouse model represents a new and robust *in vivo* paradigm for studying the pathogenesis of HD. For optical analysis, NMJs were stained with FM1-43fx and α-bungarotoxin to visualize both pre and post-synaptic elements, respectively. Confocal microscopy optical analysis showed a decrease in the number of synaptic elements and fluorescence intensity in NMJs from BACHD diaphragms compared to WT. We next analyzed pre-synaptic activity and we observed that synaptic vesicle exocytosis was impaired in NMJs from BACHD diaphragms. Ultrastructural analysis revealed significant changes in the form and sizes of the synaptic vesicles in BACHD diaphragm NMJs that could contribute to impaired exocytosis. Additionally, electrophysiology recordings revealed a decrease in the amplitude of miniature endplate potentials (MEPPs), indicating a significant reduction in the acetylcholine quantal content in synaptic vesicles from BACHD diaphragm NMJs. Our data so far suggest a function impairment in BACHD diaphragm NMJs that might occur in other muscles and then play a role in the motor defects seen in HD. These results may contribute to a better understanding of peripheral cholinergic dysfunction in this neurodegenerative disease.

Disclosures: C. Guatimosim: None. **B. Aragao:** None. **H. Rodrigues:** None. **P. Valadao:** None. **W. Camargo:** None. **L. Naves:** None. **F. Ribeiro:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.08/D11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Intravascular AAV9-EAAT2 upregulates corticostriatal EAAT2 in the Q175 mouse model of Huntington's disease

Authors: *G. V. REBEC, C. RANGEL-BARAJAS, K. D. BUNNER, S. J. BARTON; Program Neurosci & Dept. Psychological & Brain Sci.,, Indiana Univ., Bloomington, IN

Abstract: Dysregulation of glutamate transmission is a key feature of Huntington's disease (HD), a dominantly inherited condition typically characterized by progressively worsening cognitive and motor symptoms as well as dysfunction and eventual loss of neurons in corticostriatal circuits. In the striatum of HD patients and transgenic mice that model HD, there is a down regulation of the major glutamate transporter, excitatory amino acid transporter 2 (EAAT2) in humans and glutamate transporter 1 (GLT1) in mice. Reversal of this effect by systemic injection of ceftriaxone, a beta-lactam antibiotic, increases glutamate uptake and improves the behavioral phenotype in HD mice, but only for a relatively short period. A meaningful therapeutic approach to HD, however, requires a long-lasting change in EAAT2. Moreover, because the pathology is widespread throughout the corticostriatal system and may affect other brain regions as well as peripheral tissue, a global treatment strategy is required. Here, we tested the approach of using intravascular administration of adeno-associated virus serotype 9 (AAV9), which crosses the blood-brain barrier, to express EAAT2 . To determine if intravascular AAV9 can transduce cortical and striatal cells, we injected recombinant AAV9 expressing green fluorescent protein (GFP) into the tail vein of adult, heterozygous Q175 knock-in mice (HET) and wild-type (WT) controls. AAV9-GFP was diluted to a working titer of 1x1013 vg/ml and administered in a volume of 0.1 ml. At 6-8 weeks post-injection, robust transduction patterns were evident in neurons and glia in both cortical and striatal tissue of HET and WT mice. Double label immunofluorescence staining with glial fibrillary acid protein (GFAP) confirmed transduction of astrocytes, the primary cellular location of EAAT2/GLT1. To assess EAAT2 expression, HET and WT mice received tail vein injection of AAV9-EAAT2, provided by Dr. Brian Kaspar (Nationwide Children's Hospital, Ohio State University, Columbus, OH). Density analysis of cortical and striatal western blots indicated a significant increase in EAAT2 expression in both HETs and WTs relative to untreated mice. Collectively, these results validate the use of AAV9 as a delivery tool for EAAT2 up-regulation that can be part of a comprehensive treatment strategy for HD.

Disclosures: G.V. Rebec: None. **C. Rangel-Barajas:** None. **K.D. Bunner:** None. **S.J. Barton:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.09/D12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: PUCE -J13130

Senescyt-Programa Prometeo

Title: RNA interference therapy in mice subjected an experimental model of Huntington's disease

Authors: *R. AVILES REYES[1,2], D. SANCHEZ[1], S. CABRERA[1], S. ANDRADE[1], P. PALACIOS[1];
[1]Pontificia Univ. Católica Del Ecuador, Bioanalisis School, Ecuador; [2]Secretaria Nacional de Educación Superior, Ciencia, Tecnología e Innovación, Quito, Ecuador

Abstract: Huntington's disease (HD) is a late-onset and progressive neurodegenerative disorder that is caused by aggregation of mutant huntingtin protein which contains expanded-polyglutamine and it produce effect on motor control, muscles and leads to cognitive decline and psychiatric disorders. HD is wellknown pathology associated with trinucleotide repeat expansions (CAG) caused by slippage during DNA replication. As a result of the repetitive nature of DNA sequence in these regions, there may be formation of "loop out" structures during DNA replication while maintaining complementary base pairing between the parent strand and daughter strand being synthesized. RNA interference is a biological process currently being studied as a potential therapy for Huntington's disease. RNA interference (RNAi) is a natural, selective method of turning off genes, which can be induced by the production of small interfering RNAs (siRNAs) formed by a guide strand and a passenger strand. In this study we induce neurochemical injury to inoculate 1.2 µl of Q-A solution during ten days. The lesions assessments were done after 1; 3; 6; 9 doses of neurochemical damage; other animal groups were subjected to RNAi + *in vivo* fectamine and the controls of these animals were inoculated 0.9 Saline Solution; then GFAP and HTT immunohistochemistry; Hematoxylin / Eosin staining in striatum area was evaluated to test neurons and astrocytes reaction; western blot analyses of GFAP were developed and weight body mice was assessed. The results showed decrease of neuronal number and astrogliosis prominent in striatum area, furtheremore a significant weight loss of animals subjected to neurochemical experimental model. Interestingly, the treatments inoculated RNAi, presented minor astrogliosis, increase neuronal cells and enhanced the body weight. In conclusion, this research support the evidence of RNAi theraphy is capable to interfere with development of Huntington disease.

Disclosures: R. Aviles Reyes: None. **D. Sanchez:** None. **S. Cabrera:** None. **S. Andrade:** None. **P. Palacios:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.10/D13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Striatal local field potential Activity in the Q175 knock-in mouse model of Huntington's disease during plus-maze motor activity

Authors: *C. RANGEL BARAJAS, K. D. BUNNER, S. J. BARTON, G. V. REBEC; Psychological and Brain Sciences, Program in Neurosciences, Indiana Univ., Bloomington, IN

Abstract: Huntington's disease (HD), a dominantly inherited condition characterized by progressively worsening cognitive, emotional, and motor symptoms, is caused by an expanded CAG repeat (Q) in the gene that encodes for the huntingtin protein (HHT). In knock-in (KI) mouse models, the expanded CAG repeat is contained within the native HHT, closely mimicking the genetic condition of HD patients. Using the KI mouse model with 175-CAG repeats (Q175), we studied motor performance in a rotarod task as well as local field potential (LFP) activity in the striatum, a primary target of HD pathology, during approach and exit of the choice point or center of a four-arm, plus-shaped maze. Both heterozygous (HET) and homozygous (HOM) Q175 mice showed significantly shorter latency to fall in rotarod motor performance ($p<0.001$ and $p<0.0001$, respectively) compared to wild type (WT) mice. This impairment was genotype- and age-dependent. The number of arm choices in the plus maze was dramatically lower in HOM mice relative to both WT and HET ($p<0.0001$), suggesting that HOM mice were less active during plus-maze performance. Interestingly, HET mice showed significantly lower probability of turning compared to WT or HOM mice ($p<0.05$) and the effect was age-related, suggesting that age may underlie behavioral inflexibility in HET mice. Striatal LFP activity in the maze was also differentially related to genotype. Power spectral density analysis showed an increase in theta frequency (4-7 Hz) in HOM mice compared to HET and WT in the 1s before entry into the choice point, while an increase in delta frequency (1-4 Hz) was found in HET mice compared to WT or HOM in the 1s after the turning decision. Together, these data suggest that the Q175 HD model not only shows age-related deficits in motor performance, but also modulation of striatal LFP activity in relation to turning choice in the plus maze. Support: CHDI Foundation Key words: Local field potential, plus maze, Q175 Knock-in

Disclosures: C. Rangel Barajas: None. **K.D. Bunner:** None. **S.J. Barton:** None. **G.V. Rebec:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.11/D14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Grant A-5490

Title: Role of p75 neurotrophin receptor in the Q175 mouse model of Huntington's disease

Authors: *A. WEHNER[1], A. MILEN[2], R. L. ALBIN[2], B. A. PIERCHALA[2];
[1]Biol. and Materials Sci., Univ. of Michigan-Ann Arbor, Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Huntington's disease (HD) is a dominantly-inherited neurodegenerative disorder characterized by a constellation of motor, cognitive, and psychiatric symptoms. Striatal medium spiny neurons (MSNs), one of the most affected populations, are dependent on brain-derived neurotrophic factor (BDNF) anterogradely transported from the cortex for proper function and survival. Recent studies suggest both receptors for BDNF, TrkB and p75, are improperly regulated in striata of HD patients and mouse models of HD. While BDNF-TrkB signaling almost exclusively promotes survival and metabolic function, p75 signaling is able to induce survival or apoptosis depending on the available ligand and associated co-receptor. We investigated the role of p75 in the Q175 knock-in mouse model of HD by examining the level and activation of various downstream signaling molecules, as well as receptor components and effectors that associate with p75. Additionally, we examined these same signaling pathways and histopathological changes in $Q175^{WT/WT};p75^{+/+}$, $Q175^{WT/HD};p75^{+/+}$, $Q175^{WT/HD};p75^{-/-}$, and $Q175^{WT/WT};p75^{-/-}$ mice to determine if p75 and/or TrkB represent promising therapeutic targets. In $Q175^{WT/HD};p75^{+/+}$, we see an increase in activation of both Akt and NFkB in the striatum at 5 months of age compared to $Q175^{WT/WT};p75^{+/+}$, and this increase is lost in $Q175^{WT/HD};p75^{-/-}$. We see no change in activation of apoptotic pathway signaling at 5 months of age in any genotype examined. Additionally, $Q175^{WT/HD};p75^{-/-}$ show a marked decrease in DARPP-32 expression in the striatum both by western at 5 months of age and by immunohistochemistry at 7 months of age. This decrease is not seen in $Q175^{WT/HD};p75^{+/+}$. Our data suggest that p75 may play an early role in augmenting pro-survival signaling during striatal development, and disruption of this signaling may exacerbate pathology in Q175 mice.

Disclosures: **A. Wehner:** None. **A. Milen:** None. **R.L. Albin:** None. **B.A. Pierchala:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.12/D15

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS57722

the CHDIF

The Methodist Hospitals Endowed Professorship in Neuroscience

Title: Abnormalities in the dendritic branching of cholinergic interneurons and their thalamostriatal input in the Q140 knock-in mouse model of Huntington's disease

Authors: *Y. DENG, A. REINER;
Univ. of Tennessee HSC, Memphis, TN

Abstract: Premanifest HD individuals are mildly impaired in the initiation and/or execution of motor tasks. The basis of these symptoms is uncertain, as little or no neuron loss in the motor striatum has been reported in premanifest HD. Loss of cerebral and striatal white matter and reduced striatal activation during behavioral tasks has been reported, suggesting that input loss may contribute to early symptoms. In a prior study, we found that thalamostriatal axodendritic terminals are lost as early as 1 month of age in heterozygous premanifest Q140 HD mice, i.e. prior to striatal neuron loss (Deng et al., Neurobiol Dis 2013). As cholinergic interneurons are a prominent target of thalamic axodendritic terminals, we examined the thalamic input to striatal cholinergic interneurons in heterozygous Q140 males at 1 and 4 month. We used VGLUT2 immunolabeling to identify thalamostriatal terminals, and choline acetyltransferase (ChAT) immunolabeling to identify cholinergic interneurons. Stereological neuron counts showed that cholinergic perikarya were normal in abundance in Q140 mice, while Sholl Analysis revealed that their dendritic arborizations were fewer and their dendritic length was significantly decreased. Consistent with the LM evidence of dendritic arbor loss, EM analysis revealed that the abundance of ChAT+ dendritic profiles per unit area was also decreased significantly in Q140 striata. EM double-label studies showed that the abundance of VGLUT2+ axodendritic terminals making synaptic contact with ChAT+ dendrites per unit area of striatum was decreased by 40% in Q140 mice at 1 and 4 months. The loss of dendritic territory was commensurate with the overall loss of thalamic terminals on cholinergic dendrites. Thus, the density of the thalamic input to cholinergic neurons was largely unaltered in Q140 mice, although the number of terminals having input to any one neuron appeared to be less. These changes in thalamic input to striatal cholinergic interneurons may yield reduced excitatory drive to them. Physiological data on the influence of cholinergic interneurons on striatal direct versus indirect pathway neurons suggest this deficiency could contribute to early motor abnormalities (Smith et al., J Neurosci 2011).

Disclosures: Y. Deng: None. **A. Reiner:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.13/D16

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R21 NS071024

Taube Philanthropies

Koret Foundation

NS069375-01A1

Title: A small molecule p75 neurotrophin receptor ligand reduces Huntington's disease phenotypes in R6/2 and BACHD mice

Authors: *D. A. SIMMONS[1], N. P. BELICHENKO[1], S. SEMAAN[1], E. FORD[1], M. MONBUREAU[2], S. AIYASWAMY[1], C. M. HOLMAN[1], C. CONDON[1], M. SHAMLOO[2], S. M. MASSA[3,4], F. M. LONGO[1];
[1]Neurol. and Neurolog. Sci., [2]Inst. for Neuro-Innovation and Translational Res., Stanford Univ., Stanford, CA; [3]Neurol. and Lab. for Computat. Neurochemistry and Drug Discovery, [4]Dept. of Veteran's Affairs Med. Ctr. and Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Huntington's Disease (HD) is caused by an expanded polyglutamine tract in the huntingtin protein. Mutant huntingtin triggers numerous degenerative processes including loss of neurotrophic signaling, primarily attributed to reduced brain-derived neurotrophic factor (BDNF) signaling via its TrkB receptor, and increased deleterious signaling, to which the p75 neurotrophin (NTR) receptor contributes importantly. p75NTR signaling can be initiated by neurotrophins, including BDNF, and can foster either death or survival depending on the cellular context and presence of Trk receptors. Striatal p75NTR is increased while TrkB receptors are decreased in HD patients and mice and this skewed ratio was shown to up-regulate p75NTR's degenerative signaling. This increased p75NTR signaling has been shown to mediate the structural and functional synaptic plasticity deficits in HD. Thus, p75NTR is an enticing therapeutic target for HD, especially if its degenerative signaling is inhibited while its pro-survival signaling is increased. These desired effects are produced by a first-in-class small molecule p75NTR ligand, LM11A-31, developed in our laboratories. To evaluate the effectiveness of LM11A-31 against HD-related behavioral deficits and neuropathology, we administered LM11A-31 (50 mg/kg, oral gavage, once daily, 5-6 days/week) to R6/2 and BACHD mice. LM11A-31 restored striatal Akt signaling while normalizing increased PTEN, reduced the area and/or number of huntingtin aggregates in the striatum, cortex and hippocampus, and extended the survival of R6/2 mice by 18% compared to those given vehicle. The compound decreased inflammation in the striatum of both R6/2 and BACHD mice and reduced deficits in dendritic spine density of striatal medium spiny neurons and hippocampal CA1 pyramidal neurons. R6/2 mice treated with LM11A-31 showed improvements in motor behavior in an activity chamber and in their home cage. Furthermore, gait disturbances, anxiety-like behavior, and deficits in associative learning and memory were ameliorated by LM11A-31 treatment in BACHD mice. Taken together these results provide the first validation that p75NTR, a receptor critically positioned in survival and neurodegenerative signaling, might serve as an effective therapeutic target for HD. LM11A-31 has successfully completed Phase I safety and pharmacokinetic clinical trials in normal subjects and is therefore a viable candidate for HD clinical testing.

Disclosures: **D.A. Simmons:** None. **N.P. Belichenko:** None. **S. Semaan:** None. **E. Ford:** None. **M. Monbureau:** None. **S. Aiyaswamy:** None. **C.M. Holman:** None. **C. Condon:** None. **M. Shamloo:** None. **S.M. Massa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of North Carolina, University of California, San Francisco. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PharmatrophiX, University of North Carolina, University of California, San Francisco.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.14/D17

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Retinal degeneration in Huntington's disease

Authors: *M.-S. LIN[1,2], Y. CHERN[1,2];
[1]Inst. of Biomed. Science, Academia Sinica, Taipei City, Taiwan; [2]Natl. Yang Ming Uinervisity, Taipei City, Taiwan

Abstract: Huntington’s disease (HD) is the neurodegenerative disease resulted from abnormal CAG-expended Huntingtin (htt) gene. The accumulation of mutant Huntingtin protein (mHTT) is associated with neuronal death in the striatum and cortex, and subsequently give rise to multiple syndromes including motor dysfunction, and cognitive and memory impairment. Besides the impairment in the brain, HD-related pathology in the retina is less investigated and understood. Retinal dysfunction and synaptic remodeling were reported earlier in a HD mouse model (R6/1). However, the major type of retinal neurons affected by mHTT during disease progression, and the underlying pathway for such retinal dysfunction in HD remains unclear. In the present study, longitudinal changes of photosensitive neurons (the Rod and cone photoreceptors and the intrinsically photosensitive retinal ganglion cells) and non-photosensitive interneurons were observed. We also characterized the possible involvement of several important transcription factors in the retinal gene regulatory network using a HD mouse model (R6/2). Our results showed that at the late stage of HD (12 weeks old), the expression of rhodopsin was relocated to rod photoreceptor cytosol in the outer nuclear layer (ONL). Progressive decreases in the transcripts of rhodopsin and rod arrestin were also observed. Importantly, cone photoreceptors developed a more severe and earlier degenerative phenotype than rod photoreceptors. The level of S-opsin, M-opsin, and cone arrestin transcripts were down-regulated earlier than rod genes during disease progression. Besides photoreceptor, Brn3a(+) RGCs and rod bipolar cells was slightly affected in R6/2 retina. Interestingly, CRX, Nrl, Nr2e3 and TRβ2, important

transcription factors for photoreceptor differentiation, are progressive decreased in R6/2 retina compared to other transcription factors related to late cell fate determined. Collectively, we demonstrated that the photosensitive neurons are vulnerable to the expression of HD, and dysregulation of gene-regulatory network may attribute to this retinal neuropathy of HD.

Disclosures: **M. Lin:** None. **Y. Chern:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.15/D18

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Investigating behavior-related changes in striatal firing patterns in the Q175 knock-in mouse model of Huntington's disease

Authors: ***K. D. BUNNER**[1], C. RANGEL-BARAJAS[2], B. M. MCCORMICK[2], S. J. BARTON[2], G. V. REBEC[2];
[1]Program in Neurosci. & Dept. Psychological & Brain Sci., [2]Program in Neuroscience, Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Huntington's disease (HD) is a genetically inherited neurodegenerative disorder characterized by progressively worsening cognitive, emotional and motor symptoms. While the mutated huntingtin protein is found throughout the brain, the main neuropathology involves the dorsal striatum and cerebral cortex. Several animal models have been developed to improve our understanding of HD progression, and each animal model has a different neurological time course making it important to determine behavioral as well as neuronal changes corresponding to the early and/ or late stages of the disease. In this study, nest-building and open-field behavior were assessed in both homozygous (HOM) and heterozygous (HET) Q175 mice compared to wild-type (WT) controls. Nest building was assessed weekly and open field was assessed monthly starting at 28-30 weeks of age. Both the amount of building materials used and quality of the nest was significantly decreased in HOM and HET mice compared to WT controls, with HETs occupying an intermediate position. In the open field, HOMs were significantly less active than HETs as well as WT controls. Chronically implanted micro-wire bundles were used to make simultaneous assessments of open-field spiking patterns in dorsal striatum. We found a significant decrease in burst firing in both HOMs and HETs relative to WT. Our results indicate an age-related progression of multiple neurobehavioral signs in the Q175 model that parallel our findings in both truncated (R6/2) and full-length (YAC and BACHD) mouse models of HD. Moreover, preliminary analysis of our age-progression data in HET mice indicate a change in

striatal electrophysiology before overt symptom expression, suggesting that striatal dysfunction may precede onset of the subsequent behavioral phenotype.

Disclosures: **K.D. Bunner:** None. **C. Rangel-Barajas:** None. **B.M. McCormick:** None. **S.J. Barton:** None. **G.V. Rebec:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.16/D19

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01NS078008

Title: Respiratory activity and calcium uptake capacity in brain mitochondria from R6/2 mice, a model of Huntington's disease

Authors: *J. HAMILTON[1], J. J. PELLMAN[1], T. BRUSTOVETSKY[1], N. BRUSTOVETSKY[1,2];
[1]Pharmacol. and Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by severe motor and cognitive abnormalities. HD pathogenesis is linked to a mutation in a protein called huntingtin that is normally involved in developmental processes. In healthy individuals, the N-terminus of huntingtin possesses a polyglutamine stretch containing less than 35 glutamines. The mutant huntingtin (mHtt) protein has an elongated polyglutamine stretch at the N-terminus that correlates with the development of HD. The mechanism by which mHtt causes HD is unknown. Some investigators previously reported that mHtt suppresses mitochondrial respiratory activity and decreases mitochondrial Ca2+ uptake capacity. However, other investigators have found neither an impairment in respiration of isolated mitochondria and neurons derived from HD mice nor a decrease in Ca2+ uptake capacity in mitochondria isolated from the brains of HD mice. In the present study, we investigated the effect of mHtt fragments containing a 160 glutamine stretch on respiration and Ca2+ uptake capacity of mitochondria isolated from R6/2 mice, a model of HD. To confirm the presence of the elongated polyglutamine stretch in mHtt fragments, we genotyped every mouse. We evaluated respiratory activity in isolated, purified synaptic and non-synaptic brain mitochondria from 6-8 week old, symptomatic R6/2 mice and age-matched wild-type (WT) mice. We also investigated the effect of mHtt fragments on Ca2+ uptake capacity in synaptic and non-synaptic brain mitochondria isolated from R6/2 mice and WT littermates. In addition, we evaluated respiration in cultured striatal neurons derived from postnatal day 1 R6/2 mice and their WT littermates using Seahorse

XF24 flux analyzer. Mitochondrial Ca2+ accumulation was also assessed in cultured striatal neurons from R6/2 mice and their WT littermates. This has been done by evaluating FCCP-stimulated release of mitochondrial Ca2+ into the cytosol following Ca2+ loading into mitochondria during glutamate-induced increase in cytosolic Ca2+. In experiments with isolated mitochondria, we found no difference in respiratory activity and Ca2+ uptake capacity between mitochondria from R6/2 mice and WT animals. Experiments with cultured striatal neurons demonstrated similar respiratory activity and mitochondrial Ca2+ accumulation comparing neurons derived from R6/2 and WT mice. Thus, our data do not support mHtt-mediated impairment of mitochondrial respiratory activity and Ca2+ uptake capacity assessed either in isolated brain mitochondria or in cultured striatal neurons from R6/2 mice.

Disclosures: J. Hamilton: None. **J.J. Pellman:** None. **T. Brustovetsky:** None. **N. Brustovetsky:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.17/D20

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Novo Nordisk Foundation

Title: Human glia can both induce and rescue aspects of disease phenotype in Huntington's disease

Authors: A. BENRAISS[1], S. WANG[1,3], S. HERRLINGER[1], X. LI[1], D. CHANDLER-MILITELLO[1], J. P. MAUCERI[1], H. B. BURM[1], *M. J. TONER[2], Q. XU[1], F. DING[1], F. WANG[1], N. KANG[1], J. KANG[4], M. S. WINDREM[1], I. MUNOZ-SANJUAN[5], M. NEDERGAARD[1,3], S. A. GOLDMAN[1,3];
[1]Ctr. for Translational Neuromedicine, [2]Neurol., Univ. of Rochester, Rochester, NY; [3]Ctr. for Basic and Translational Neurosci., Univ. of Copenhagen, Copenhagen, Denmark; [4]Dept. of Anat. and Cell Biol., New York Med. Col., Valhalla, NY; [5]CHDI Fndn., Princeton, NJ

Abstract: Glial pathology has been noted to contribute to a broad set of neurodegenerative and neuropsychiatric diseases traditionally considered disorders of solely neuronal dysfunction. Huntington's Disease is a prototypic neurodegenerative disorder, characterized by abnormally long CAG repeat expansions in the first exon of the Huntingtin gene. To define the contribution of glial pathology to Huntington's disease (HD), we established human HD glial chimeras by neonatally engrafting the striata of immunodeficient mice with mutant huntingtin (mHTT)-expressing glial progenitor cells (GPCs), derived from huntingtin mutant human embryonic stem

cells (hESC) or mHTT-transduced human forebrain hGPCs. Mice engrafted with mHTT-expressing (48Q) hESC GPCs manifested significantly worse motor performance than controls chimerized with normal (18Q) hESC GPCs. To assess the basis for this effect, we established human glial chimeras using human fetal striatal GPCs transduced to express the first exon of mutant HTT (73Q and 23Q), and patch-clamped local medium spiny neurons (MSNs). MSNs in the mHTT glial environment manifested higher input resistance, less frequent spontaneous EPSPs, and lower excitation thresholds than those resident with normal (23Q) HTT-transduced glia. We then asked if the converse manipulation, engraftment of normal glia into an HD environment, might slow disease progression in R6/2 (120Q) HD mice. R6/2 mice engrafted with normal hGPCs survived longer than unengrafted R6/2s, and manifested slower motor deterioration, while their MSNs exhibited lower input resistance, more frequent spontaneous EPSPs, and were less excitable. Furthermore, whereas R6/2 mice manifested abnormally high levels of interstitial potassium, chimerization with normal glia restored striatal potassium levels to near normal. These observations suggest a causal role for glia in HD, and indicate that the colonization of diseased striata with wild-type glia may slow disease progression.

Disclosures: **A. Benraiss:** None. **S. Wang:** None. **S. Herrlinger:** None. **X. Li:** None. **D. Chandler-Militello:** None. **J.P. Mauceri:** None. **H.B. Burm:** None. **M.J. Toner:** None. **Q. Xu:** None. **F. Ding:** None. **F. Wang:** None. **N. Kang:** None. **J. Kang:** None. **M.S. Windrem:** None. **I. Munoz-Sanjuan:** None. **M. Nedergaard:** None. **S.A. Goldman:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.18/D21

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI

Title: Nucleus accumbens dopamine release and motivated behavior are compromised in the Q175 mouse model of Huntington’s disease

Authors: *D. P. COVEY[1], H. M. DANTRASSY[2], I. GILDISH[2], J. F. CHEER[2,3];
[2]Anat. and Neurobio., [3]Psychiatry, [1]Univ. of Maryland, Baltimore, MD

Abstract: Huntington’s disease (HD) is a fatal, neurodegenerative disease caused by a mutation in the HTT gene. Motor abnormalities, including chorea, akinesia and dystonia, represent the classic symptoms that characterize HD. Additionally, cognitive and emotional disturbances affecting memory, attention, executive function and mood are commonly described non-motor symptoms and these may present early in disease progression before the onset of motor abnormalities. Imaging studies in humans and measures of dopamine release in animal HD

models indicate disrupted striatal dopaminergic function as a common manifestation of HD that may precede motor deficits. However, a direct association between compromised but temporally resolved dopamine release measurements and behavior in HD is lacking. Here, we assessed alterations in dopamine release and reward seeking in the heterozygous Q175(+/-) knock-in mouse HD model. Dopamine was monitored in the nucleus accumbens using fast-scan cyclic voltammetry while mice lever pressed for sucrose reward on a fixed interval (FI) 30 s and progressive-ratio (PR) schedule to assess timing and motivation, respectively. Both strains of mice showed similar patterns of responding on the FI task, indicating that timing is not disrupted in this mouse HD model. However, Q175(+/-) mice displayed significantly lower breakpoints on the PR task relative to wild-type controls. Impaired motivation was associated with a decrease in the peak dopamine signal evoked by reward receipt. Genotypic differences in dopamine release increased as trials progressed and cost increased and were significantly different during the final trials of the PR session as animals reached their breakpoint. Moreover, while a strong correlation was observed between peak reward-evoked dopamine release and breakpoint in wild-type controls, no correlation was observed in Q175(+/-) mice, indicating that lower levels of subsecond dopaminergic activity accompany the pursuit of reward. These findings indicate that compromised transduction at dopamine receptors in the nucleus accumbens may contribute to cognitive behavioral deficits, particularly decreased motivation that are commonly observed in HD prior to phenoconversion.

Disclosures: **D.P. Covey:** None. **H.M. Dantrassy:** None. **I. Gildish:** None. **J.F. Cheer:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.19/D22

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Marie Curie PIIF-GA-2011-300197

Title: Further evidence of defective sphingolipid metabolism across multiple HD animal models*

Authors: A. DI PARDO, E. AMICO, M. FAVELLATO, S. SCIACCA, F. SQUITIERI, *V. MAGLIONE;
IRCCS Neuromed, Pozzilli, Italy

Abstract: Huntington's disease (HD), the most common dominantly inherited neurodegenerative disorder affecting an estimated 3 to 7 per 100000 people, is characterized by the progressive striatal and cortical neurodegeneration and associated motor, cognitive and behavioral disturbances. The disease causing mutation is an expansion of CAG trinucleotide repeats (>36

repeats) encoding a polyglutamine (polyQ) stretch in N-terminal region of huntingtin (Htt), a ubiquitous protein whose function is still unclear. Expansion of the polyQ stretch endows mutant Htt (mHtt) with toxic properties and results in the development of a broad array of cell dysfunctions including dysregulation of gene expression, reduced synthesis and release of neurotrophins (i.e. BDNF) as well as impaired lipid homeostasis. We have recently demonstrated that metabolism of sphingolipids (gangliosides) is impaired in HD and seems to play a critical role in the susceptibility of neuronal cell to apoptosis. Defective sphingolipid metabolism has been reported also in different other neurodegenerative conditions like Alzheimer and Parkinson diseases supporting the hypothesis that sphingolipid alterations may be critical in the pathogenesis of the neurodegenerative disorders. In this study we investigated the content of different sphingolipids and the expression of some sphingolipid metabolic enzymes in multiple brain area of two different HD animal models. Our findings consolidate and extended the evidence that sphingolipid metabolism is aberrant in multiple HD models. Importantly, we also showed that defects in lipid homeostasis, in particular reduced levels of gangliosides, are detected early in the disease even before any sign of pathology appears in both animal models. Collectively our data clearly suggest that defective sphingolipid metabolism might potentially represent the molecular basis of severe structural and functional alterations normally associated with the disease.

Disclosures: **A. Di Pardo:** None. **E. Amico:** None. **M. Favellato:** None. **S. Sciacca:** None. **F. Squitieri:** None. **V. Maglione:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.20/D23

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI

Title: Decrease in maximal, not basal, CMRO2 in R6/2 mouse model of Huntington's disease by ultra-high field 17O magnetic resonance spectroscopy

Authors: ***J. M. DUBINSKY**[1], S. LOU[2], T. LEPAK[2], W. CUI[3], X.-H. ZHU[3], G. OZ[3];
[1]Dept Neursci, [2]Neurosci., [3]Ctr. for Magnetic Resonance Res., Univ. of Minnesota, Minneapolis, MN

Abstract: *In vivo* evidence for brain mitochondrial dysfunction in animal models of HD is scarce. We used the novel 17O magnetic resonance spectroscopy (MRS) technique on R6/2 mice to directly determine rates of oxygen consumption (CMRO2) and mitochondrial function *in vivo*. Maximal CMRO2-max, represents the maximum flux through all of metabolism, from glucose

through electron transport. *In vitro*, maximal respiration is proportional to the concentration of cytochrome oxidase in electron transport and can be used as a measure of mitochondrial capacity. We compared *in vivo* basal and maximal CMRO2 in the presence of dinitrophenol (DNP), using 16.4 T 17O MRS in male HD mice and littermates at 9wk. In addition, we examined the amount respiration decreased in the presence of oligomycin to block oxidative phosphorylation. Utilizing 17O MRS (Cui et al, 2013 JCBFM), the basal oxygen consumption rate did not differ between isoflurane anesthetized R6/2 mice and littermates. At rest, striatal CMRO2-basal of R6/2 mice was 2.49±0.35 μmol/g/min (mean ± stdev, N=9), while that of littermates was 2.67±0.33 μmol/g/min (N=10), indicating comparable mitochondrial output despite onset of motor symptoms in R6/2. CMRO2-max was tested 10 min after systemic injection of DNP, an uncoupler that shunts the proton electrochemical gradient producing maximal mitochondrial respiration. After DNP injection, the maximal CMRO2 in both striatum and cortex of R6/2 mice was significantly lower than that of control mice, indicating a lower spare energy generating capacity. In a separate set of mice, oligomycin injection decreased CMRO2 equally in brains of R6/2 and wild type mice, suggesting oxidative phosphorylation capacity was equivalent at rest. When examining expression levels of representative mitochondrial proteins from *ex vivo* tissue samples, VDAC/actin was significantly higher in striatum than in cortex in WT mice, but not in R6/2. No significant differences were observed in individual electron transport protein expression with respect to VDAC or actin, although trends were noted. A similar pattern was observed for regional differences in cytochrome oxidase activity, with striatal activity exceeding cortical activity in wild type, but not R6/2. In determinations of total oxidized regional proteins by Oxyblot, R6/2 cortical protein oxidation may exceed that of wild type. Together, these data suggest that the R6/2 decrease in striatal CMRO2 may be attributed to a decrease in mitochondria or mitochondrial function and that the cortical CMRO2 decrease may result from constraints upstream in energetic pathways.

Disclosures: **J.M. Dubinsky:** None. **S. Lou:** None. **T. Lepak:** None. **W. Cui:** None. **X. Zhu:** None. **G. Oz:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.21/D24

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

NIH PP-AG14930

Burke Medical Research Institute

Title: Nine reactions to powerhouse abnormalities in Huntington's disease

Authors: ***N. NASERI**[1], H. XU[2], J. BONICA[2], J. G. VONSATTEL[3], E. P. CORTES[3], L. C. PARK[4], J. ARJOMAND[4], G. E. GIBSON[2];
[1]Neurosci., Weill Cornell Med. Col., New York City, NY; [2]Burke Med. Res. Inst., Weill Cornell Med. Col., White Plains, NY; [3]New York Brain Bank at Columbia Univ., New York City, NY; [4]CHDI Fndn., Los Angeles, CA

Abstract: Glucose metabolism is reduced in the brains of patients with Huntington disease (HD). The mechanisms underlying this deficit, its link to the pathology of the disease, and the vulnerability of the striatum in HD remain unknown. Abnormalities in some of the key mitochondrial enzymes involved in glucose metabolism, including the pyruvate dehydrogenase complex (PDHC) and the tricarboxylic acid (TCA) cycle, may contribute to these deficits. Here, activities for these enzymes and select protein levels were measured in human postmortem cortex and in striatum and cortex of an HD mouse model (Q175); mRNA levels encoding for these enzymes were also measured in the Q175 mouse cortex. The activities of PDHC and nearly all of the TCA cycle enzymes were dramatically lower (-50% to 90%) in humans than in mice. The activity of succinate dehydrogenase increased with HD in human (35%) and mouse (223%) cortex. No other changes were detected in the HD cortex or mouse striatum. In Q175 cortex, there were increased activities of PDHC (+12%) and aconitase (+32%). Increase mRNA levels for succinyl thiokinase (+88%) and isocitrate dehydrogenase (+64%) suggested an upregulation of the TCA cycle. These patterns of change differ from those reported in other diseases, which may offer unique metabolic therapeutic opportunities for HD patients.

Disclosures: **N. Naseri:** None. **H. Xu:** None. **J. Bonica:** None. **J.G. Vonsattel:** None. **E.P. Cortes:** None. **L.C. Park:** None. **J. Arjomand:** None. **G.E. Gibson:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.22/D25

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH RO1 GM071775 to A.B

NIH RO1 GM105781 to A.B

NIH RO1 GM112179 to A.B

NIH/NINDS R01 NS064269 to G.R.Z

University of Miami Neuroscience Center

Pew Charitable Trust

Title: Mitochondrial biogenesis attenuates polyQ-induced proteotoxicity in yeast, fly, and human cell models of Huntington's disease

Authors: *A. RUETENIK[1], A. OCAMPO[2], K. RUAN[3], K. RING[5], L. M. ELLERBY[5], G. R. ZHAI[3], A. BARRIENTOS[4];
[1]Neurosci. Program, [2]Dept. of Biochem. and Mol. Biol., [3]Dept. of Mol. and Cell. Pharmacol., [4]Dept. Biochem. and Mol. Biology, Dept. of Neurol., Univ. of Miami, Miller Sch. of Med., Miami, FL; [5]Buck Inst. for Res. on Aging, Novato, CA

Abstract: Abnormalities in mitochondrial function and metabolism are a recurring feature in a wide range of neurodegenerative disorders. Diseases in which these alterations have been documented include diseases caused by the misfolding of proteins with expanded polyglutamine (polyQ) tracts, which include nine known inherited neurological disorders, including Huntington's disease (HD). These disorders are thought to cause neurodegeneration through the toxic-gain-of function of the abnormal protein, and disease severity is closely linked to the number of glutamine repeats. We and others have shown that maintenance of mitochondrial fitness attenuates polyQ-induced proteotoxicity and have shown evidence that suggests that human huntingtin exon 1 gene fragments expressing 103 repeats of polyQ (hHttExQ103) associates with the mitochondrial outer membrane causing mitochondrial dysfunction. However, the complex interplay between mitochondrial function, polyQ toxicity, and disease progression remains to be fully understood. To investigate these questions in a simple setting we have created yeast models of polyQ diseases that allow for the induced expression of specific toxic proteins during yeast chronological lifespan (CLS), a model of neuronal aging. Our results show that in these models, toxicity is significantly attenuated by the enhancement of mitochondrial biogenesis by protecting cellular oxidative phosphorylation (OXPHOS) without altering polyQ oligomerization. Toxicity is also attenuated by caloric restriction, which increases mitochondrial respiration and resistance to stress. To confirm these results in a multicellular organism, we have used a *Drosophila* model of Huntington's disease created by overexpressing exon 1 of the human huntingtin gene (hHttExQ93) with 93 repeats of polyQ. In these models, enhancement of mitochondrial biogenesis by overexpression of DmPGC-1/spargel in the nervous system with a pan-neuronal driver significantly reduced neuronal apoptosis and improved climbing performance at all ages. We have also found similar results in human neuronal stem cells derived from human HD-patient fibroblasts. Our results suggest that therapeutic approaches protecting mitochondrial respiration could reduce polyQ toxicity and delay the development of clinical symptoms in patients.

Disclosures: **A. Ruetenik:** None. **A. Ocampo:** None. **K. Ruan:** None. **K. Ring:** None. **L.M. Ellerby:** None. **G.R. Zhai:** None. **A. Barrientos:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.23/D26

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS46742

Title: Genome-wide DNA methylation in mouse models of Huntington's disease: impact of super-enriched environment

Authors: *J.-Y. HWANG[1], M. SUZUKI[2], K.-M. NOH[1], B. L. COURT VAZQUEZ[1], J. M. GREALLY[3], R. S. ZUKIN[1];
[1]Dept Neurosci, Albert Einstein Col. Med., Bronx, NY; [2]Dept. of Genet. and Med., [3]Dept. of Genet. and Medicine, Dept. of Pathology, Abert Einstein Col. of Med., Bronx, NY

Abstract: Huntington's disease (HD) is an inherited, late onset neurodegenerative disorder characterized by progressive motor, psychiatric and cognitive decline. Marked neuronal loss occurs in the cortex and striatum. HD is caused by a polyglutamine (CAG) expansion in the 5' coding region of the gene encoding huntingtin. Environmental enrichment has been shown to delay the onset and progression of motor symptoms and to improve neurological function and cognitive performance in animal models of HD. Epigenetic modifications such as DNA methylation and histone modification are critical to genome reprogramming during brain development, tissue-specific gene expression and global gene silencing in adult mice. Recent findings implicate epigenetic modifications in the progressive neurodegeneration associated with a number of brain diseases. However, the impact of enriched environment on DNA methylation in a transgenic mouse model of HD is, as yet, unclear. Here we show the impact of enriched environment on DNA cytosine methylation in the striatum of R6/2 mice by means of an unbiased, genome-wide screen for abnormal DNA methylation using HELP-tagging, a next generation, quantitative DNA methylation assay. HD and wild-type mice were reared in either a stark or super-enriched environment. Under conditions of stark environment, DNA methylation in the striatum of HD mice was modestly increased relative to that of wild-type littermates. In contrast, striatal DNA from HD mice exposed to a super-enriched environment exhibited overall hypomethylation vs. wild-type mice reared in the same conditions. Bioinformatic analysis reveals that genes exhibiting hypomethylation include those involved in synaptic function and plasticity and/or brain development. We predict that identification of genes and gene networks with altered DNA methylation status in HD will help identify novel targets for therapeutic intervention and accelerate development of novel therapeutic strategies to ameliorate motor and cognitive deficits associated with this debilitating and devastating disease.

Disclosures: J. Hwang: None. **M. Suzuki:** None. **K. Noh:** None. **B.L. Court Vazquez:** None. **J.M. Greally:** None. **R.S. Zukin:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.24/D27

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R01 NS082338 (to W.D)

Intramural Research Program of the National Institute on Drug Abuse (to Y.Y and H. L)

Title: Imaging intrinsic connectivity networks in a full-length huntingtin knock-in mouse model

Authors: *T. REN, SR[1,2], H. LU[6], Q. LI[3], P. QI[2], J. ZHANG[3], Y. YANG[6], W. DUAN[2,4,5]; [1]Emergency, Beijing Tiantan Hosp. Capital Med. Universit, Beijing, China; [2]Div. of Neurobiology, Dept. of Psychiatry & Behavioral Sci., [3]Radiology,, [4]Neurosci., [5]Program in Cell. and Mol. Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [6]Neuroimaging Res. Br., Natl. Inst. on Drug Abuse, NIH, Baltimore, MD

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease, which results in progressive neuronal degeneration in the neostriatum and neocortex, and associated functional impairments in motor, cognitive, and psychiatric domains. To date, proven neuroprotective strategies remain elusive although there has been a rapid progress in the understanding of the pathogenetic mechanisms. Part of the problem has been that most of the trials have attempted intervening at a time when the degenerative process is already far advanced and hence when it would be difficult even for the most effective therapy to demonstrate any benefit. Through genetic testing, people who will ultimately develop HD can be identified before clinical onset, raising the possibility of initiating therapy in this prodromal period to delay or prevent disease onset. In order to accurately assess the effectiveness of disease-modifying therapies in a group of clinically normal HD carriers, biomarkers that reflect the early cell dysfunction have become of paramount importance. Resting state functional MRI (RS-fMRI) constitutes a novel paradigm that examines spontaneous brain activity in the absence of task, and it enables us to investigate the functional connectivity between brain regions. We conducted a preliminary RS-fMRI functional connectivity study of a full-length huntingtin knock-in mouse model. Independent component analysis of RS-fMRI data identified spatially consistent brain networks in mice as those reported in rats. Further seed-based functional connectivity analysis demonstrated altered network-related activity in selected areas of the default mode network in HD mice, such as reduced connectivity between motor cortex and striatum, as well as between anterior cingulate cortex and thalamus. These preliminary results suggest that a specific subset of brain networks is altered in these HD mice, and encourage further investigation on whether the resting state network changes are representing effects of ongoing neurodegeneration, and whether the altered resting state networks are correlated with behavioral abnormality and synaptic dysfunction in HD. These results, if confirmed with a large cohort of mice, could

potentially provide insights into elucidating the cellular mechanisms responsible for perturbed brain network connectivity, as well as evaluating potential therapeutic interventions in HD.

Disclosures: **T. Ren:** A. Employment/Salary (full or part-time):; Beijing Tiantan Hospital, Capital Medical University. **H. Lu:** A. Employment/Salary (full or part-time):; National Institute on Drug Abuse, NIH. **Q. Li:** A. Employment/Salary (full or part-time):; Johns Hopkins University School of Medicine. **P. Qi:** A. Employment/Salary (full or part-time):; Johns Hopkins University School of Medicine. **J. Zhang:** A. Employment/Salary (full or part-time):; Johns Hopkins University School of Medicine. **Y. Yang:** A. Employment/Salary (full or part-time):; National Institute on Drug Abuse, NIH. **W. Duan:** A. Employment/Salary (full or part-time):; Johns Hopkins University School of Medicine.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.25/D28

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation Inc. Grant

CIHR Grant GPG-102165

Title: Loss of the Huntington's disease-associated palmitoylacyltransferase HIP14 in adulthood leads to death due to progressive paralysis and seizures, motor and psychiatric disturbances, and astrogliosis and microglial activation

Authors: *S. S. SANDERS[1], M. P. PARSONS[2], A. L. SOUTHWELL[1], K. K. N. MUI[1], S. FRANCIOSI[1], L. A. RAYMOND[2], M. R. HAYDEN[2];
[1]Ctr. for Mol. Med. and Therapeut., [2]Dept. of Psychiatry and Brain Res. Ctr., The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Huntington disease (HD) is caused by a CAG expansion in *HTT* and is characterized by striatal atrophy and motor, cognitive, and psychiatric symptoms. The interaction between mutant HTT and HIP14 (zDHHC17), a palmitoyl acyltransferase for HTT, is disturbed, resulting in reduced palmitoylation of HTT and other HIP14 substrates. *Hip14*-deficient mice recapitulate many features of HD, including striatal atrophy and motor deficits. However, the phenotype is developmental, unlike in HD mice and patients. A model of post-developmental loss of HIP14 was generated to examine the role of HIP14 in neurological deficits and neurodegeneration. This mouse model allows for highly efficient *Hip14* deletion upon tamoxifen administration at 6-weeks of age ($iHip14^{\Delta/\Delta}$). $iHip14^{\Delta/\Delta}$ mice show dramatically reduced survival due to progressive paralysis and seizures beginning 10 weeks after loss of *Hip14.* At 3 months (~7 weeks after loss of *Hip14*) $iHip14^{\Delta/\Delta}$ mice have motor deficits, anhedonia, and increased escape response.

Electrophysiological analysis suggests that *iHip14*$^{\Delta/\Delta}$ mice have striatal dysfunction and an imbalance between excitatory and inhibitory synapses in the hippocampus. *iHip14*$^{\Delta/\Delta}$ mice have increased cortical volume due to early and very striking astrogliosis and microglial activation suggesting a novel role for HIP14 in glial cells. Interestingly, mice deficient in any one of the following palmitoylated HIP14 interacting proteins or HD-related proteins have similar phenotypes to *iHip14*$^{\Delta/\Delta}$ mice: CSP, GLUA2 Q/R editing, KCNMA1, SLC1A3, and Kir4.1. The palmitoylation levels of these proteins will be assessed in *iHip14*$^{\Delta/\Delta}$ mice. This indicates that loss of *Hip14* from conception allows for developmental compensation that cannot take place if *Hip14* deficiency occurs in the adult.

Disclosures: S.S. Sanders: None. **M.P. Parsons:** None. **A.L. Southwell:** None. **K.K.N. Mui:** None. **S. Franciosi:** None. **L.A. Raymond:** None. **M.R. Hayden:** None.

Poster

303. Huntington's Disease *In vivo*

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 303.26/D29

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR Grant MOP-84438

CIHR Doctoral Research Award

UBC 4-Year Fellowship

Title: Investigating the effects of conditional caspase-6 deficiency on the YAC128 mouse model of Huntington's disease

Authors: *S. LADHA, D. E. EHRNHOEFER, B. K. Y. WONG, P. RUDDLE, Q. XIA, Y. DENG, D. CHEUNG, S. FRANCIOSI, M. R. HAYDEN;
Univ. of British Columbia, Ctr. For Molecul, Vancouver, BC, Canada

Abstract: Huntington disease (HD) is an autosomal dominant neurodegenerative disorder characterized by motor, cognitive, and psychiatric symptoms. HD is caused by a CAG repeat expansion in the huntingtin (HTT) gene resulting in the production of mutant huntingtin (mHTT). Caspase-6 is a cysteine aspartyl protease that plays a central role in apoptosis and has been implicated in several neurodegenerative diseases. Increased caspase-6 activation is observed in early grade human HD brains and in mouse models of HD and our lab has previously demonstrated that inhibiting caspase-6-mediated cleavage of mutant huntingtin protects against neuropathology and behavioural deficits in a mouse model of HD. Constitutive genetic ablation of caspase-6 in the YAC128 mouse model of HD results in a partial rescue of some features of HD; however, the continued presence of the 586 fragment suggests possible

developmental reprogramming and compensation for caspase-6 function. In order to circumvent this, we are investigating the effects of the genetic loss of caspase-6 in adulthood on the pathogenesis of HD, a strategy that more closely mimics a clinical therapy. To that end, we have created an inducible caspase-6 knockout/YAC128 mouse using the Cre-loxP recombination system. Knockout was induced in male and female mice at six weeks of age by daily intraperitoneal injections of 100mg/kg of tamoxifen for 19 days. This treatment paradigm results in ~50% loss of caspase-6 in the brain and 95-100% loss of caspase-6 in the peripheral tissues. Behavioural characterization of these mice reveals no change in rotarod performance but a delay in the climbing deficit and an improvement in the anxiety phenotype as assessed by the elevated plus maze. Neuropathological assessement shows no differences in striatal volume loss and a significant reduction in corpus callosum volume in mice lacking caspase-6 compared to YAC128 controls. Peripherally, the complete loss of caspase-6 attenuates the overactive inflammatory response observed in YAC128 mice and delays body weight gain. These data suggest that partial loss of caspase-6 in the brain is not sufficient to improve most behavioural and neuropathological phenotypes; however, an assessment of peripheral phenotypes following complete loss of caspase-6 in non-CNS tissues points to an important role for caspase-6 in mediating peripheral inflammation and body weight gain in HD.

Disclosures: **S. Ladha:** None. **D.E. Ehrnhoefer:** None. **B.K.Y. Wong:** None. **P. Ruddle:** None. **Q. Xia:** None. **Y. Deng:** None. **D. Cheung:** None. **S. Franciosi:** None. **M.R. Hayden:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.01/D30

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CONACyT grants No. 138663

CONACyT grants No.129303

Title: Effect of resveratrol on neuronal dendritic morphology of medial prefrontal cortex and dorsal hippocampus in spontaneously hypertensive rats

Authors: *H. COATL CUAYA[1], L. M. DE JESÚS[1], A. D. DÍAZ[2], R. A. VÁZQUEZ-ROQUE[1], M. J. GÓMEZ-VILLALOBOS[1], G. FLORES[1];
[1]Inst. de Fisiología, [2]Facultad de Ciencias Químicas, Benemerita Univ. Atonoma De Puebla, Puebla, Mexico

Abstract: Hypertension arterial (HTA) is a main risk factor for development cardiovascular disease, cerebrovascular disease and renal failure, which are considered a public health. Reports

indicate that HTA can damage the brain, inducing a brain vascular disease associated with cerebral ischemia in patients at long-term. Consequently this may affect cognitive functions. The cerebrovascular disease affect the functioning of the limbic system, which regulates different both motor and cognitive functions. Besides several reports suggests that prefrontal cortex (PFC) and hippocampus play an important role in the development of the processes of learning and memory. Recent studies indicate that in a model of HTA, which is the spontaneously hypertensive rat (SH), there are some alteration in the dendritic morphology in brain regions such as PFC and hippocampus CA1. This is due to the release of free radicals associated with endothelial damage that induce the development of oxidative stress, which is responsible for neuronal death. It is suggested that the use of antioxidants such as resveratrol, may help prevent oxidative stress and this neuronal damage in a process of HTA, however, this is not yet clear. The aim was to evaluate the effect of administration of resveratrol on the dendritic morphology of the prefrontal cortex and hippocampus of spontaneously hypertensive rats. We use SH male rats of 15 months, which were administered orally with resveratrol (50mg/Kg/day) during 8 weeks. After the brain was removed and processed for Golgi-Cox stain method, to perform morphological analysis of the PFC (layer III and V) and dorsal hippocampal CA1 region. The results of neuronal morphological analysis in PFC and dorsal hippocampus show a neuroprotective effect that prevents alterations induced by HTA, through promote an increase in the dendritic tree for some areas analyzed. The most obvious increase was observed in the dendritic length in PFC; layer III and V, and dorsal hippocampus CA1 region and dentate gyrus. This suggest that resveratrol exerts a neuroprotective effect against the alterations caused by the chronic course of the HTA. (Supported by: CONACyT grants No. 138663 and 129303 to G Flores).

Disclosures: **H. Coatl Cuaya:** None. **L.M. De Jesús:** None. **A.D. Díaz:** None. **R.A. Vázquez-Roque:** None. **M.J. Gómez-Villalobos:** None. **G. Flores:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.02/D31

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Interfering with progranulin degradation to combat frontotemporal dementia

Authors: *X. ZHOU, Y. WEN, E. GIBBS, W. JIA, M. S. CYNADER;
UBC hospital, Brain Res. Ctr. Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Mutations in progranulin (PGRN) have been found to be a common cause of familial FTLD and the major cause of FTLD with tau-negative ubiquitin positive inclusions. Most mutations result in decreased PGRN expression and PGRN haplo-insufficiency is strongly

associated with FTLD. Sortilin (SORT1), a member of vacuolar-protein-sorting-10-protein (VPS10) family, recently has been found to be a PGRN binding partner. When PGRN binds to the SORT1 receptor, it is rapidly endocytosed and transported to lysosomes for degradation (Hu et al. 2010). Overexpression of SORT1 in cultured HeLa cells dramatically reduces PGRN levels in conditioned media, whereas knockdown of SORT1 increased extracellular PGRN levels. This SORT1 mediated PGRN level increase was also observed in SORT1 knockout mice(Hu et al. 2010). Thus, SORT1-mediated PGRN endocytosis is likely to play a central role in FTLD-TDP pathophysiology. Rather than knocking-out SORT1, we are using small interference peptides to block PGRN binding to the SORT1 receptor on this rapid degradation pathway in order to maintain extracellular PGRN levels. Our preliminary studies using peptide arrays has shown that PGRN can bind to multiple sites on SORT1. Based on the potential binding sites, we generated several deleted mutations of SORT1. To determine the critical PGRN binding domains on SORT1, we used Biacore to analyse real-time binding affinity between those mutants SORT1 with PGRN. Interestingly, one of the binding sites is located in the same domain bound by other pro-neurotrophins such as pro-BDNF and pro-NGF. We speculate that binding of PGRN to SORT1 may prevent apoptosis induced by pro-NTs, through competitive binding. This speculation may explain the requirement of certain levels of PGRN to maintain a healthy brain. Here, we aim to define PGRN binding sites on SORT1, assess the role of this complex in cell death, and create peptide antagonists to block the PGRN- SORT1 interaction as a therapeutic strategy to raise PGRN levels in FTLD.

Disclosures: **X. Zhou:** None. **Y. Wen:** None. **E. Gibbs:** None. **W. Jia:** A. Employment/Salary (full or part-time):; University of British Columbia. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; University of British Columbia. **M.S. Cynader:** A. Employment/Salary (full or part-time):; University of British Columbia. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; University of British Columbia.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.03/D32

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Pick's disease: characterization of pathology with diverse stains

Authors: *J. BAUN, S. HUTCHINGS, J. BALLARD, C. ZURHELLEN, B. TIPTON, J. KIEFFER, R. C. SWITZER, III;
Neurosci. Associates, Knoxville, TN

Abstract: Pick's Disease (PiD) is a neurodegenerative disease characterized by neuronal cell death in the frontal and temporal lobes of the brain. Symptoms include dementia, changes and difficulty in speech patterns, and many behavioral indicators including anxiety, confusion, attenuated social abilities and disengagement from friends and family. Onset of the disease usually occurs between the ages of 40 and 60, with the average age of onset being 54. We evaluated the pathology post-mortem in the brain of a 68 year old female using various histological and antibody stains. One of the main indicators of the disease is a buildup of amyloid and tau proteins, which was evaluated using Ab 1-42 and AT8 (respectively) as well as other amyloid and tau markers. Several silver stains were also used to detect abnormal pathology. The Campbell-Switzer method revealed amyloid deposits and plaques, which resembled Alzheimer's (AD) pathology but the plaques were far fewer in number in the PiD brain than an average AD brain. A Silver Protein protocol (Wako, cat#283-90241), a modified Bodian stain, revealed both amyloid plaques and neurofibrillary tangles. Thionine Nissl and H&E stains showed swollen, "ballooned" neurons, one of the hallmark indicators of PiD. A hypertrophied glial state was observed with both GFAP (astrocytes) and Iba1 (microglia). The Perl's/DAB stain for ferric iron positively stained glia, and surprisingly also intensely stained many neurons. This battery of stains on near adjacent tissue sections further characterizes the unique pathology of PiD.

Disclosures: J. Baun: None. **S. Hutchings:** None. **J. Ballard:** None. **C. Zurhellen:** None. **B. Tipton:** None. **J. Kieffer:** None. **R.C. Switzer:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.04/D33

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Intramural Research Program of the National Eye Institute, NIH

Title: Myocilin protects axons from myelin-associated glycoprotein-induced degeneration

Authors: *S. I. TOMAREV[1], H. S. KWON[1], C. JAWORSKI[2], I.-H. YANG[3];
[1]SRGCB, LRCMB, [2]LRCMB, NEI, NIH, Bethesda, MD; [3]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: *Myocilin* (*MYOC*) gene encodes a secreted glycoprotein. Mutations in *MYOC* may lead to juvenile and adult-onset primary open-angle glaucoma. The main goal of this study was

to elucidate possible role of myocilin in axon growth and degeneration using dorsal root ganglion (DRG) and retinal explant cultures as well as *Myoc* null mice.Sequencing of RNA isolated from 2 month-old optic nerves of wild-type and *Myoc* null mice demonstrated changes in components of the signaling pathway governing axon guidance. Addition of 1 μg/ml of purified myocilin to DRG cultures protected neurites from myelin-associated glycoprotein (MAG)-induced degeneration as well as reduced MAG-stimulated growth cone collapse. The direct physical interaction of myocilin and MAG was demonstrated by co-immunoprecipiatation from mouse optic nerve lysates. This interaction as well as the modulation of RhoA-GTPase level by myocilin may contribute to the protective effects of myocilin. Addition of myocilin to P4 mouse retinal explants similarly protected neurites from MAG-induced degeneration and reduction of neurite length. Myocilin provides protective action through interaction with soma or axons alone as was shown by cultivating DRGs in microfluidic two-compartment chambers. Addition of myocilin to the axonal compartment did not lead to changes in the number of axons or their total length compared with untreated samples, but protected axons from MAG-induced degeneration. Addition of myocilin to the axonal compartment induced changes in the levels of several mRNAs in axons as was shown first by PCR array analysis and confirmed by quantitative RT-PCR. We concluded that myocilin protects axons from MAG-induced degeneration and this protection could be achieved through interaction with axons, soma or both.

Disclosures: S.I. Tomarev: None. **H.S. Kwon:** None. **C. Jaworski:** None. **I. Yang:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.05/D34

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MIUR grant ““I meccanismi neurocognitivi alla base delle interazioni sociali” (PRIN2010XPMFW4_008)

Università degli Studi di Milano-Bicocca CARIPLO grant "Dottorato ad alta Formazione in Psicologia Sperimentale, Linguistica e Neuroscienze Cognitive"

Title: Emotion recognition in the behavioral variant of frontotemporal dementia: evidence from error patterns analysis and resting-state brain activity

Authors: *S. F. CAPPA[1,2], N. CANESSA[1,2], A. DODICH[3], C. CERAMI[3], G. LETTIERI[3], C. CRESPI[3], S. IANNACCONE[2], A. MARCONE[2], A. FALINI[3,2], D. PERANI[3,2];
[1]IUSS Pavia, Pavia, Italy; [2]San Raffaele Scientific Inst., Milan, Italy; [3]San Raffaele Univ., Milan, Italy

Abstract: Background and aims: Patients with the behavioral variant of frontotemporal dementia (bvFTD) present pervasive social dysfunctions, including deficits in emotion recognition (Diehl-Schmid et al, 2007). Previous evidence suggests a global deficit in the ability to recognize emotional cues, more pronounced for negative emotions, in these patients. This impairment has been previously associated with structural changes in fronto-temporal and limbic regions (Kumfor et al, 2013). To date no studies have investigated the relationship between emotion recognition abilities and resting state activity, which is also abnormal in bvFTD (Whitwell et al, 2011). Materials and Methods: We administered the Italian version of the Ekman 60-Faces test (Dodich et al., 2014) to 18 bvFTD patients (age=68.7±7.90 years, education=10.94±3.93 years) and 26 healthy controls (57.46±7.96 years, education=13.58±3.74 years). All subjects also underwent a functional magnetic resonance imaging (fMRI) session lasting 10 minutes, during which they lied quietly, awake with eyes closed. We used a group Independent Component Analysis (gICA), implemented in the GIFT toolbox (http://mialab.mrn.org/software/gift/), to analyze the relationship between task performance and resting-state metrics in bvFTD patients vs. controls. Results: Compared to controls, bvFTD patients showed an overall deficit in emotion recognition, as well as higher rate of confusion among negative emotions, highlighted by an error-pattern analysis. Resting-state analyses showed abnormal resting state activity in bvFTD, particularly in the anterior portion of the Default-Mode Network (DMN) and in attentional and executive networks. One of these networks, including the dorsomedial prefrontal cortex, inferior frontal gyrus and amygdala, showed a specific relationship between spectral properties and the ability to recognize negative emotions, with both reduced coherent internal activity and a stronger correlation between activity and performance. Conclusion: The analysis of specific resting-state networks represents an intermediate level of analysis between abnormal brain structure and impaired social abilities in these patients, showing an association between emotion recognition deficits and abnormal brain functioning in fronto-limbic regions.

Disclosures: **S.F. Cappa:** None. **N. Canessa:** None. **A. Dodich:** None. **C. Cerami:** None. **G. Lettieri:** None. **C. Crespi:** None. **S. Iannaccone:** None. **A. Marcone:** None. **A. Falini:** None. **D. Perani:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.06/D35

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant AG043503

NIH Grant AG017856

Wyncote Foundation

Title: Association of genetic variations and neuropsychiatric symptoms in progressive supranuclear palsy (PSP)

Authors: *K. TERNES[1], K. RASCOVSKY[1], E. M. WOOD[1], M. GROSSMAN[1], V. VAN DEERLIN[2], C. T. MCMILLAN[1];
[1]Neurolgy, [2]Ctr. for Neurodegenerative Dis. Res., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Progressive supranuclear palsy (PSP) is the second most common cause of akinetic-rigid movement disorders, and is characterized by gait disturbance and postural instability with falls, supranuclear vertical gaze palsy, and cognitive dysfunction. PSP is a tauopathy with abnormal accumulation of misfolded tau protein in neurons of the midbrain, basal ganglia, brainstem, and cerebrum, contributing to neuronal loss in these regions. While the causes of PSP are unknown, a recent genome-wide association study in autopsy-proven PSP identified 11 single nucleotide polymorphisms (SNPs) associated with increased risk of PSP. There is also clinical heterogeneity in PSP where recent clinical observations suggest a behavioral overlap with behavioral variant frontotemporal dementia (bvFTD), of which tau is the also the underlying pathology in roughly 40% of bvFTD cases. In this exploratory study we evaluate whether SNPs previously associated with PSP are associated with their behavioral features. We evaluated 41 PSP patients on 5 subscales of the Neuropsychiatric Inventory (NPI) and short-form NPI-Q that are typically associated with bvFTD. Our cohort included 34 clinically-diagnosed PSP patients who have highly-probable PSP pathology and 9 autopsy-confirmed PSP patients. Analysis revealed evidence of behavioral changes on all 5 evaluated NPI subscales: apathy (74%), disinhibition (29%), irritability (50%), appetite (44%), and aberrant motor behavior (27%). We then examined genetic associations in 32 of the 43 PSP patients who were genotyped using a customized panel for the 11 PSP GWAS SNPs. Assuming a dominant model of inheritance, we observed an association between rs2493013 (EXOC2) and an increased risk of disinhibition (LR=7.623; p=0.006) and aberrant motor behavior (LR=7.623; p=0.006). We also observed an association between minor allele carriers in rs6687758, an intergenic SNP in Chromosome 1, and an increased risk of apathy (LR=10.08; p=0.001): 100% of homozygous major allele carriers did not exhibit apathetic behavior. All other SNPs did not achieve an association corrected for multiple comparisons. Together, these findings suggest that two SNPs, rs2493013 and rs6687758, may confer an increased risk of behavioral and psychiatric phenotype in PSP. We suggest that future work is required to evaluate the potential genetic risk factors and mechanisms that contribute to the clinical heterogeneity observed in PSP.

Disclosures: K. Ternes: None. **K. Rascovsky:** None. **E.M. Wood:** None. **M. Grossman:** None. **V. Van Deerlin:** None. **C.T. McMillan:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.07/D36

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: FWO Grant G090915N

Title: Progranulin deficiency delays nerve regeneration and functional recovery following nerve crush injury

Authors: S. BEEL[1], L. DE MUYNCK[1], L. M. J. VAN DEN BOSCH[1], W. L. ROBBERECHT[2], *P. VAN DAMME[2];
[1]Lab. of Neurobiology, Vesalius Res. Center, VIB, Leuven, Belgium; [2]Neurol. Department, UZ Leuven, Leuven, Belgium

Abstract: Frontotemporal dementia (FTD) is the second most common form of early-onset dementia, after Alzheimer's disease. Symptomatic onset of this primary progressive, neurodegenerative disorder is most common between the ages of 40 to 60 years and can be clinically subdivided into one behavioral and two language variants. About 40% of the patients have a familial history and one of the most common forms of familial FTD is caused by loss of function mutations in the gene encoding progranulin (GRN). These mutations lead to a reduction of 50% or more of GRN levels in the blood and cerebrospinal fluid. Therefore, haploinsufficiency seems to be the mechanism of disease in these patients. GRN is a pleiotropic growth factor and its expression in the CNS is limited to neurons and microglia. *In vitro* studies already showed the importance of GRN in neuronal survival and axonal outgrowth. However, an *in vivo* setting would allow for a much better approach to unravel the mechanisms by which GRN exerts its neurotrophic functions in the CNS. Therefore, we generated GRN knockout mice and established a nerve crush model to study axonal regeneration and functional recovery in the absence or presence of GRN. By comparing our crush model to an axotomy model, using the sciatic nerve, we confirmed that our crush injury induces complete degeneration of the distal nerve segments which is important to ensure homogeneous injury across all animals. To study the effect of GRN depletion on nerve regeneration after injury, we performed a crush on the facial nerve of young GRN knockout mice (8 - 12 weeks). Functional recovery was checked on a daily basis by comparing the whisker movement on the injured side to the movement of the contralateral non-injured side and scoring the movement on a scale from 0 to 3 (0 = no recovery; 1 = detectable motion; 2 = significant (but asymmetric) voluntary motion of all whiskers; 3 = symmetric voluntary motion of all whiskers). GRN knockout mice showed a significant delay in recovery compared to non-transgenic littermate controls. Crossbreeding GRN knockout mice with human GRN (hGRN) overexpressing mice made it possible to study if hGRN can substitute for the endogenous mouse GRN. Indeed, the overexpression of hGRN in a knockout background showed a complete rescue of the delayed whisker movement recovery of GRN knockout mice. These observations suggest that GRN is an essential component in the nerve regenerative process and that hGRN can mediate these effects. Further research will be necessary to unravel the precise mechanism underlying this *in vivo* neurotrophic effect of GRN.

Disclosures: S. Beel: None. **L. De Muynck:** None. **L.M.J. Van Den Bosch:** None. **W.L. Robberecht:** None. **P. Van Damme:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.08/D37

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Effect of genetic background on the phenotype of chorea-acanthocytosis model mouse

Authors: *H. SAKIMOTO, O. NAGATA, S. YOKOTSUKA, K. ARAI, M. NAKAMURA, A. SANO;
Kagoshima Univ., Kagoshima-Shi, Japan

Abstract: Chorea-acanthocytosis (ChAc) is an autosomal recessive neurodegenerative disorder that is caused by loss of function mutations in the VPS13A gene. Clinically, ChAc is characterized by acanthocytosis in erythrocytes and Huntington disease like various neuropsychiatric symptoms that indicate the existence of phenotypic modifiers. In the previous sturdy, we previously produced ChAc model mice encoding a human disease mutation with deletion of exons 60-61 in Vps13a by gene targeting. The mutant mice that have a hybrid C57BL/6J and 129/Sv genetic background displayed variable phenotypes, strongly suggesting the existence of modifier genes. We backcrossed the ChAc model mice separately onto C57BL/6J, 129S6/Sv, DBA/2J, BALB/c, and FVB/N genetic backgrounds. Osmotic fragility test of erythrocytes and measurement of body weight were performed for these mice. We found a marked increase in the osmotic fragility of red blood cells in the ChAc mutant mice of 129S6/Sv, FVB/N, and C57BL/6J. Statistically significant low body weight against weeks of age was found in ChAc mutant model mouse of FVB/N, 129S6/Sv, and DBA/2J backgrounds. Subsequently, we performed behavioral analyses for the mice of the low body weight strains. The observed abnormal behavioral phenotypes in the ChAc model mice varied according to the strain backgrounds. These results suggest that there may be modifying factors of ChAc symptoms in genes that generate genetic background of each mouse strain.

Disclosures: H. Sakimoto: None. **O. Nagata:** None. **S. Yokotsuka:** None. **K. Arai:** None. **M. Nakamura:** None. **A. Sano:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.09/D38

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIMH 5T32MH019118-23 (SL)

Title: Motor-related neuronal activity in cerebellar thalamus of Essential Tremor patients

Authors: *D. J. SEGAR[1], S. LEE[2], S. R. JONES[2], W. F. ASAAD[2];
[2]Neurosci., [1]Brown Univ., Providence, RI

Abstract: Introduction: The ventral intermediate nucleus of the thalamus (VIM), a key link between the cerebellum and the sensorimotor cortex, has long been a neuromodulatory target for pathologic tremor. Stimulation of VIM can dramatically reduce tremor in these patients, but the underlying mechanisms by which VIM modulates motor output are poorly understood. Methods: We obtained single neuron recordings in VIM from Essential Tremor (ET) patients during deep brain stimulation (DBS) electrode implant surgery. To assess neuronal responses to movement direction, patients performed a center out joystick task in which they manipulated a joystick to move a cursor on a screen toward a target. Targets appeared individually at one of eight locations around a circle. On some trials, the target jumped to a predictable or a random location just after initiation of movement. Spike responses were recorded at 40 kHz, thresholded, and sorted offline using principal components analysis. Results: Each neuron was tested for task-related responsiveness by comparing baseline firing rates to firing rates cued to particular task-related events using a non-parametric bootstrap. Approximately half of all neurons responded to at least one task-related visual cue or movement, and several were selectively responsive to specific visual cues or movements. Some neurons demonstrated similar responses to both visual and motor events, suggesting that they may be involved in the visual-motor transformation. Other neurons exhibited classical "tuning" to movements in specific directions. A small group of neurons responded significantly more to the appearance of a "jumped" target and the associated movement correction. These neurons may be directly related to the transmission of a motor error correction signal. Conclusions: VIM neurons were actively engaged by our center out joystick movement task, with a substantial number tuned to the direction of the patient’s movement. A few also selectively responded to corrections of movement, supporting a role for the VIM in conveying alterations in motor plans from the cerebellum to the cortex.

Disclosures: D.J. Segar: None. **S. Lee:** None. **S.R. Jones:** None. **W.F. Asaad:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.10/D39

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: IUT Grant IUT20-41

Title: Anxiety in Wfs1-deficient mice is probably related to changes in brain cells' ability to cope with endoplasmic reticulum stress

Authors: *A. ALTPERE, S. SÜTT, S. RAUD, R. REIMETS, M. LOOMETS, E. VASAR; Univ. of Tartu, Tartu, Estonia

Abstract: Mutations in WFS1 (Wolfram syndrome 1) gene are associated with rare hereditary disorder called Wolframin syndrome that presents with diabetes, nerve atrophy and several psychiatric disorders. This condition is associated with impaired cell survival, but the exact mechanism of how Wfs1 affects cell survival remains unknown. Wfs1 has been associated with changes in expression level of several endoplasmic reticulum (ER) stress related genes in cell cultures. Prolonged ER stress in turn is associated with cell death. In this study we investigated how Wfs1-deficiency affects mRNA expression of ER stress related genes in brain of naive mice and those that were exposed to elevated plus maze. Namely, we analyzed mRNA expression levels of Grp78, Grp94, total and spliced Xbp1, Atf6α, Chop and Perk in ventral striatum, temporal lobe, hippocampus and prefrontal cortex. We found that there were no differences in mRNA expression level of ER stress related genes in naive Wfs1-deficient mice compared to wild type mice. Difference in expression level of ER stress related genes in brain of Wfs1-deficient mice compared to wild type mice became apparent only after exposure to elevated plus maze and was region specific, with most differences in ventral striatum. For example in ventral striatum mRNA expression levels of Chop and spliced Xbp1 were significantly lower in Wfs1-deficient mice compared to wild type mice after elevated plus maze. Expression level on Grp94 in ventral striatum was significantly higher in Wfs1-deficient mice after exposure to plus maze when compared to naive Wfs1-deficient mice. No such changes were found in ventral striatum of wild type mice. Spliced Xbp1 is shown to be negative regulator of ER stress related apoptosis. No change in spliced Xbp1 mRNA expression after stressful situation may suggest that Wfs1-deficiency alters cells' ability to cope with ER stress and results in apoptosis.

Disclosures: A. Altpere: None. **S. Sütt:** None. **S. Raud:** None. **R. Reimets:** None. **M. Loomets:** None. **E. Vasar:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.11/D40

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Miracles for MSA

Barefoot Runners of Dayton, Ohio

Title: Increased presence of inflammatory proteins within the pontocerebellar tracts in Multiple System Atrophy

Authors: A. B. VALENTI[1], W. T. BOOTHBY-SHOEMAKER[1], B. KATBAMNA[2], *C. F. IDE[1];
[1]Biol. Sci., [2]Speech Pathology and Audiol., Western Michigan Univ., Kalamazoo, MI

Abstract: Multiple System Atrophy (MSA) is an adult onset progressive neurodegenerative disease, characterized by various degrees of Parkinsonism, cerebellar ataxia and autonomic failure. In MSA, the protein alpha-Synuclein (α-Syn) forms misfolded protein aggregates that appear primarily in oligodendrocytes in the white matter tracts of the pons and cerebellum. In other synucleinopathies such as Parkinson's disease, the accumulation of α-Syn is associated with microglial activation, production of inflammatory cytokines and chemokines, and T-cell infiltration (Lee et al., 2010; Brochard et al. 2009). However, little is known about an inflammatory and adaptive immune response in MSA. Previous Affymetrix studies conducted by our lab revealed upregulation of genes involved in an immune response in MSA pons (Langerveld et al, 2007). To further define changes in pons tissue related to MSA, immunohistochemistry (IHC) was performed on transverse sections from MSA and control pons tissues from the New York Brain Bank at Columbia University. IHC analysis showed the upregulation of CD33 (expressed on microglia; associated with the impairment of microglia-mediated clearance of amyloid-beta in Alzheimer's Disease), CD68 (marker of microglia and macrophages), and CD8α (involved in adaptive immune response; cytotoxic T cell marker) in three regions of the pontocerebellar tracts. Our pilot study revealed an increasing trend of CD68 and CD33 expression in the dorsal-most, mid-, and ventral pontocerebellar fiber tracts in MSA pons. The data also showed a significant increase of CD8α expression ($p=0.003$) in the dorsal-most, mid-, and ventral pontocerebellar fiber tracts. These data suggest inflammation may contribute to the neuropathology that occurs in MSA, and that CD33 may limit microglia-mediated clearance of protein aggregates in MSA.

Disclosures: A.B. Valenti: None. **W.T. Boothby-Shoemaker:** None. **B. Katbamna:** None. **C.F. Ide:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.12/D41

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Dorsal genital nerve stimulation reduces incontinence episodes in idiopathic OAB patients

Authors: *J. DONG[1], A. M. RYHAMMER[2], N. J. M. RIJKHOFF[1];
[1]Hlth. Sci. and Technol., Aalborg Univ., Aalborg Ost, Denmark; [2]Dept. of Urology, Pelvic Floor Unit, Aarhus Univ. Hospital, Skejby, Aarhus, Denmark

Abstract: Purpose: Different treatment methods for overactive bladder (OAB) are available, including fluid advice, bladder training, pharmacotherapy, neuromodulation and surgical treatment. Neuromodulation (sacral or tibial nerve stimulation) was established on reducing the symptoms of OAB. However, the drawbacks limit their wide clinical application. Therefore, a new neuromodulation is needed. Previous experiments on animals have revealed a lasting suppression of the parasympathetic micturition reflex after stimulation of the dorsal genital nerve (DGN) 1. The aim of this study is to assess the effect of DGN stimulation on incontinence episodes (IE) using surface electrodes in idiopathic OAB patients. Methods: Two idiopathic OAB patients were recruited. Stimulation was performed using a battery powered handheld stimulator in patient's home. A monophasic square constant current pulse with a width of 200 μs and a frequency of 20 Hz was used. One electrode was placed on the clitoris, and the second electrode was placed 3-4 cm lateral to the clitoris. More details of stimulation can be seen in the study by Worsøe et al. 2. Stimulation was performed twice daily for 15 minutes in each session for a period of 3 weeks. All data (frequency, severity of urgency (none, mild, moderate, severe), number of IE per day) were collected during 4 days baseline pre-stimulation, a 3 weeks home test and 4 days post-stimulation. Results: Until now, two female patients have completed the experiment. The reduction from pre-stimulation to post-stimulation in average IE per day was 86% and 57% respectively. The average IE per day post-stimulation was lower than the average IE during and pre-stimulation in these two patients. Conclusions: Reduction of average IE per day was seen in both patients. If more positive data can be obtained from enough patients, DGN stimulation using surface electrodes might be a potential therapeutic option in management of idiopathic OAB in women resistant to conservative treatment. Acknowledgements: The authors wish to thank continence nurse, Tina Schwennesen from Pelvic Floor Unit, Aarhus University Hospital, Skejby, who provided assistant work in the experiment. Reference: 1. Jiang CH, Lindstrom S. Prolonged enhancement of the micturition reflex in the cat by repetitive stimulation of bladder afferents. J Physiol. 1999; 517 (2):599-605. 2. Worsøe J, Fynne L, Laurberg S, Krogh K, Rijkhoff NJM. Electrical stimulation of the dorsal clitoral nerve reduces incontinence episodes in idiopathic faecal incontinent patients: a pilot study. Colorectal Dis. 2012; 14(3):349-355.

Disclosures: J. Dong: None. **A.M. Ryhammer:** None. **N.J.M. Rijkhoff:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.13/D42

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Low dose aspirin as a prophylactic against the progression of Scrapie in Mice

Authors: *D. N. BRYANT[1], M. A. BENNEYWORTH[2], D. M. SEELIG[3];
[1]Vet. Clin. Sci., Univ. of Minnesota, Saint Paul, MN; [2]Mouse Behavior Core, Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN; [3]Vet. Clin. Sciences, Col. of Vet. Med., Univ. of Minnesota, St. Paul, MN

Abstract: Transmissible Spongiform Encephalopathies (TSEs) are a group of infectious neurodegenerative diseases that occur in mammals. Our current understanding is that TSE progression involves the conversion of the benign Prion protein (PrP^{C}) into its harmful and infectious counterpart (PrP^{SC}). Increased PrP^{SC} production is associated with a number of consequences, including: PrP^{SC} aggregation, stimulation of pro-apoptotic signaling, gliosis, motor deficits, cognitive deficits and death. Since there is no known cure for TSEs, we wanted to test the hypothesis that acetylsalicyclic acid (ASA or Aspirin) is a primary or secondary prophylactic against the progression of the TSE known as Scrapie. ASA has been shown to protect against PrP^{SC} toxicity *in vitro* via its antiapoptotic effects. C57BL 6 mice were divided into 4 groups of 6-8 mice (males and females). 1. ASA pretreatment, 2. ASA Intervention at behavioral onset of symptoms, 3. scrapie only, 4. No ASA/No scrapie. Group 1 was administered ASA (30ug/ml ad libitum in drinking water) two weeks prior to a single intraperitoneal injection of scrapie inoculum (ME7, 100ul of 5%) to groups 1,2 and 3. Mice remained on ASA for the duration of the study. Since there is little to no data on early motor/behavioral deficits associated with scrapie progression, we performed three behavior assays (Pole Test for bradykinesia, Forelimb Grip Strength and Rotarod for motor coordination) monthly. Baseline motor behavior was assayed after group 1 started ASA but prior to inoculation. Preliminary data suggest that scrapie mice are beginning to show a deficit on the pole test at 52 days post inoculation. Compared to control animals, scrapie mice have increased latency to turn and reach the bottom of the pole. No trend or difference was observed in the Rotarod or grip strength test as of 52 days post inoculation. The progression of scrapie takes approximately 200-250 days in this mouse model system. We will continue to monitor the effect of ASA on motor behavior and survival. ASA intervention will occur when we have statistically significant confirmation of a motor deficit. Finally, we will harvest brains, confirm PrP^{SC} expression and assay apoptotic signaling pathways in terminal mice.

Disclosures: D.N. Bryant: None. **M.A. Benneyworth:** None. **D.M. Seelig:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.14/D43

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Behavioral and sperm motility analyses of male infertility in Chorea-acanthocytosis model mouse

Authors: *O. NAGATA, H. SAKIMOTO, S. YOKOTSUKA, K. ARAI, M. NAKAMURA, A. SANO;
Kagoshima Univ., Kagoshima Univ., Kagoshima-Shi, Japan

Abstract: Chorea-acanthocytosis (ChAc) is a rare autosomal recessive neurodegenerative disorder caused by loss of function mutations in the vacuolar protein sorting 13 homolog A (VPS13A) gene that encodes chorein. It is characterized by adult-onset chorea, erythrocyte acanthocytosis, and neuropsychiatric symptoms. We previously produced a ChAc model mouse introducing exons 60-61 deletion corresponding to a human disease mutation by a gene targeting technique. The ChAc model mice exhibited male infertility. In the present study, we performed analyses of mating behavior, olfactory behavior, sperm count, and sperm motility in order to elucidate a cause of the male infertility. Sperm motility was siginificantly decreased in ChAc model mice compared with littermate contorols although mating behavior, olfactory behavior, and sperm count analyses showed no significant differences. These results suggests that the male infertility of the ChAc model mouse is caused by asthenozoospermia. Further studies are needed to clarify a molecular role for chorein in sperm motility and its association with ChAc molecular pathogenesis.

Disclosures: O. Nagata: None. **H. Sakimoto:** None. **S. Yokotsuka:** None. **K. Arai:** None. **M. Nakamura:** None. **A. Sano:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.15/D44

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Fulbright Fellowship

Title: Role of yeast Hsp31/DJ1 in Sup35 prion aggregation

Authors: *K. ASLAM, T. HAZBUN;
Purdue Univ., West Lafayette, IN

Abstract: Amyloids are a biochemically well-characterized state of protein aggregation commonly associated with neurodegenerative diseases in mammals and cause heritable traits in *Saccharomyces cerevisiae*. An associated class of amyloid aggregation state includes prions, which are self-replicating, misfolded proteins capable of adopting amyloid aggregates in cells.

Sup35, a translation-termination factor, is one of the original and best-studied prions in yeast. Yeast provides a useful model to study amyloid aggregation including Sup35 prion formation. Yeast Hsp31 is a member of small heat shock proteins that has structural similarity with human DJ1 protein. Previously, we have showed that Hsp31 possesses chaperone properties with protective effects against α-Syn toxicity in yeast. In the present study, we elucidate the mechanism of Hsp31 modulation of prion formation in yeast. We find that Hsp31 inhibits the formation of Sup35 aggregates as determined by fluorescence microscope, flow cytometry and SDD-AGE. Furthermore, overexpression of Hsp31 transiently inhibits the induction of Sup35 prion but has no significant effect over prolonged induction and cannot cure the [PSI^+] prion state. We hypothesize that Hsp31 acts on the early stages of prion formation therefore, it can't cure already formed aggregates. In fact, localization of Hsp31 under fluorescence microscope showed that Hsp31 and Sup35 aggregates do not co-localize; rather Hsp31 is occluded from Sup35 prion aggregates, indicating that Hsp31 acts on substrates prior to the formation of large aggregates. We also find that deletion of HSP31 in [PSI^+] background modulates the level of curing by Hsp104. Further studies are investigating the possible interaction of Hsp31 with early prion and α-Syn oligomers to establish further mechanistic insight in the biological roles of the Hsp31/DJ-1 chaperone family.

Disclosures: K. Aslam: None. **T. Hazbun:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.16/D45

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIMH 5T32MH019118-23

NSF DMS-1042134

Title: Intraoperative recording and computational modeling of modulation of tremor related oscillations in human ventral intermediate nucleus of the thalamus

Authors: *S. LEE[1], D. J. SEGAR[1], W. F. ASAAD[2], S. R. JONES[2];
[2]Neurosci., [1]Brown Univ., Providence, RI

Abstract: Essential Tremor (ET) is the most common movement disorder and is typically characterized by a 4-8 Hz intention tremor, manifested upon voluntary movement toward a target. For severe, medication refractory ET, deep brain stimulation (DBS) of the cerebellar motor thalamus, the ventral intermediate nucleus (VIM), can be an effective treatment to alleviate tremor. The causes and pathophysiology of ET and the mechanisms by which DBS alleviates tremor in these patients are not fully understood. We are integrating intraoperative

electrophysiological recording from VIM in human ET patients performing a center out motor task with biophysically principled computational modeling of cerebellar-thalamic-cortical circuits to help understand the pathophysiology of ET and mechanisms of effective DBS. During DBS lead implant surgery, we recorded extracellular field potential and single unit spike activity from VIM while patients used a joystick to move a cursor on a screen toward a target. The target appeared at one of eight locations on a circle, and upon initiation of movement, the target sometimes jumped to another location in either a predictable or random manner. Patients were required to move the cursor to the target and hold at the target until cued for the end of the trial. We measured reaction times, time to final target, and distance from final target trajectory to quantify performance on the task and correlate with neurophysiology and tremor. We quantified tremor frequency (4-8 Hz) field activity consistent with the tremulous movement and observed increases in tremor activity during movement and maintenance of posture, which was modulated differently for random and predictable trials. Finally, we developed a computational model of cerebellar input into VIM consisting of multicompartment thalamocortical relay (TC) cells, thalamic relay nucleus (TRN) cells, and inhibitory thalamic interneurons to understand how differences in cerebellar input to VIM may modulate tremor activity, with implications for tremulous motor output. In the model, we find that timing and strength of the inhibitory inputs, coupled with T-type and L-type calcium channels in TC and TRN cells, affect how tremor oscillatory activity is manifested in VIM. We discuss the implications of our work for understanding the effects of DBS. Together, our joint data and modeling provides novel detail on multiple aspects of movement-related activity in VIM.

Disclosures: **S. Lee:** None. **D.J. Segar:** None. **W.F. Asaad:** None. **S.R. Jones:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.17/D46

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Beat Batten Foundation

Sanford Health

NIH Grant P20 GM103620

NIH Grant P20 GM103548

NIH Grant R01NS082283

Title: Suppressing an infantile neuronal ceroid lipofuscinosis associated nonsense mutation

Authors: *R. GERAETS[1,2], J. M. WEIMER[1,2], D. A. PEARCE[1,2];
[1]Sanford Sch. of Med. - Univ. of South D, Sioux Falls, SD; [2]Children's Hlth. Res. Ctr., Sanford Res., Sioux Falls, SD

Abstract: Lysosomal storage disorders (LSD) result from deficiencies in either a lysosomal enzyme or membrane protein. A deficiency in the soluble lysosomal enzyme palmitoyl-protein thioesterase 1 (PPT1) causes the LSD known as Classic Infantile Neuronal Ceroid Lipofuscinosis (INCL). Medically, this disease is classified as a rare neurodegenerative disorder with hallmarks of retinopathy, progressive motor and cognitive decline, and premature death. Different genetic mutations in *CLN1* give rise to the PPT1 deficiency. Approximately 25% of patients with INCL have disease causing nonsense mutations in at least one allele; the most common being $CLN1^{R151X}$. The deleterious effect of nonsense mutations can be suppressed using read through compounds (RTCs). RTCs suppress nonsense mutations by promoting non-recognition of premature termination codons, resulting in the production of full length proteins. The objective of this study was to evaluate the effectiveness of RTCs at suppressing the $CLN1^{R151X}$ nonsense mutation. *In vitro* and *ex vivo* models derived from the $CLN1^{R151X}$ mouse were treated with various concentrations of the following RTCs: Gentamicin, Ataluren, RTC-13, and Amlexanox. Patient-derived, immortalized lymphoblasts containing the $CLN1^{R151X}$ mutation were also treated. Changes in PPT1 activity were assessed using a fluorogenic enzyme assay. Results indicate that select RTCs can successfully increase PPT1 activity. Collectively, our findings support further investigation into this therapeutic approach.

Disclosures: R. Geraets: None. **J.M. Weimer:** None. **D.A. Pearce:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.18/D47

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Lions International Hearing Foundation

Title: Fluctuation analysis of steady gaze in essential tremor

Authors: *J. H. ANDERSON[1,3], J. WILLGING[2], J. ASHE[4,5];
[1]Dept Otolaryngology, [2]Neurol., Univ. Minnesota, Minneapolis, MN; [3]Otolaryngology, [4]Neurol., Minneapolis VA Hlth. Care Syst., Minneapolis, MN; [5]Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Some oculomotor abnormalities suggesting cerebellar dysfunction have been identified in essential tremor. Some aspects of pursuit tracking, small amplitude saccades and vestibular reflexes have been studied. Less is known about large amplitude saccades, optokinetic

reflexes, and oscillations during steady gaze. The present study is an effort to quantify variability in eye movements and to characterize the presence of eye jerks/oscillations during steady gaze. The general aim is to identify and characterize the short and long range correlation and self-similarity of fluctuations in gaze over time using a multifractal, de-trended fluctuation analysis (Kantelhardt et al., 2002), MFDFA, of time series data. For this initial work the analysis is applied to recordings during fixation at a straight-ahead, stationary target; while attempting to maintain a straight-ahead position in complete darkness; and during optokinetic stimulation with a constant velocity visual stimulus. The preliminary results show promise for characterizing possible fractal properties of the oculomotor system dynamics, interactions across temporal scales, and how that is manifested in essential tremor. This approach could provide further insight into the underlying pathophysiology affecting the control of gaze in essential tremor.

Disclosures: J.H. Anderson: None. **J. Willging:** None. **J. Ashe:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.19/D48

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R01NS082283

P20 GM103620

P20 GM103548

Title: Morphological changes in the peripheral tissues of Neuronal Ceroid Lipofuscinosis mouse models

Authors: *R. N. LAUFMANN, D. TIMM, J. M. WEIMER;
Children's Hlth. Res. Ctr., Sanford Res., Sioux Falls, SD

Abstract: Neuronal Ceroid Lipofuscinoses (NCLs) are a family of lysosomal storage disorders characterized by visual, motor, and cognitive decline, behavioral deficits, and premature death. Pathologically, NCLs result in an accumulation of autofluorescent material in the lysosome, neurodegeneration, disruptions in lysosomal enzyme function, and robust glial activation. Initial studies from our laboratory have also suggested that, in addition to the defects within the central nervous system, other organs, including the kidney, spleen and thymus, may be severely affected in vLINCL. We hypothesize that these additional factors outside of the CNS contribute to the premature death of our mutant mice. We will present evidence of morphological differences in the periphery between mutant and wild type mice in the spleen and liver, blood analysis data, and evidence for potential biomarkers that could be used to track the progression of the disease. Our

main goal for this project is to see if there are notable differences in the periphery, so that future studies may be focused on using combinatorial treatments for both the CNS and peripheral tissues.

Disclosures: R.N. Laufmann: None. **D. Timm:** None. **J.M. Weimer:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.20/E1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant AG043503

NIH Grant AG017586

Wyncote Foundation

Title: Functional compensation for executive deficits in progressive supranuclear palsy

Authors: *C. A. OLM[1], B. M. KANDEL[2], B. B. AVANTS[2], J. A. DETRE[3], J. C. GEE[2], K. RASCOVSKY[1], M. GROSSMAN[1], C. T. MCMILLAN[1];
[1]Penn Frontotemporal Degeneration Center, Dept. of Neurol., [2]Penn Image Computing and Sci. Lab, Dept. of Radiology, [3]Dept. of Radiology and Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Progressive supranuclear palsy (PSP) is a neurodegenerative disorder characterized by postural instability, supranuclear gaze palsy and other visual system problems, and cognitive or behavioral deficits. While PSP is most commonly characterized as a movement disorder with primary midbrain atrophy, frontal cortical atrophy has also commonly been observed along with accompanying frontally mediated executive dysfunction. However, PSP is clinically heterogeneous with some patients having more severe executive deficits while others have relatively spared cognitive function. In this study we evaluate whether functional mechanisms may provide compensatory support for executive deficits in PSP and account for some of the previously reported heterogeneity. To evaluate whether there may be functional mechanisms for executive compensation in PSP we performed a multimodal assessment of gray matter using cortical thickness measured with T1 MRI and cortical cerebral blood flow (CBF) measured with pseudo-continuous arterial spin labeling (pCASL). We evaluated 17 clinically-diagnosed PSP patients and 19 demographically-comparable healthy controls. We used a verbal fluency task in PSP patients as a measure of executive function, involving the generation of as many words as possible in one minute that begin with the letter “F”. Based upon the T1 MRI we analyzed cortical thinning in patients relative to controls. From the pCASL images, we calculated CBF

that was corrected for partial voluming to control for cortical thinning. Cortical thinning was found in bilateral inferior and prefrontal cortex and extended to include right insula and superior temporal cortex. Regions of hypoperfusion were widespread throughout frontal cortex and extended to include temporal and parietal cortices. Regions showing hypoperfusion minimally overlapped with regions of reduced cortical thickness as expected since this analysis controlled for gray matter/CSF and gray matter/white matter partial voluming effects. A regression analysis relating verbal fluency performance to corrected CBF revealed two clusters in which higher CBF within bilateral ventrolateral and inferior frontal cortex was associated with better executive function. Together, these findings suggest that increased perfusion in the frontal cortex is associated with increased performance on an executive task and these findings are consistent with other studies implicating these regions in verbal fluency tasks. We interpret this as evidence of functional compensation and suggest that compensatory mechanisms in PSP may account for some of the heterogeneity of cognitive impairments reported in this disease.

Disclosures: **C.A. Olm:** None. **B.M. Kandel:** None. **B.B. Avants:** None. **J.A. Detre:** None. **J.C. Gee:** None. **K. Rascovsky:** None. **M. Grossman:** None. **C.T. McMillan:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.21/E2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: HIAE, IIEP1796-13

Title: The contralateral cortical silent period is not abnormal in primary Restless Legs Syndrome / Willis-Ekbom disease

Authors: *A. CONFORTO[1], G. DO PRADO[2], E. AMARO JR.[3], A. ECKELI[4], S. MAGALHÃES[3];
[1]Hosp. Das Clínicas/São Paulo Univ. and Inst. Israelita De Ensino E P, Sao Paulo, Brazil; [2]Univ. Federal de São Paulo, São Paulo, Brazil; [3]Hosp. Israelita Albert Einstein, São Paulo, Brazil; [4]Univ. de São Paulo - Ribeirão Preto, Ribeirão Preto, Brazil

Abstract: Background: Restless legs syndrome (RLS) is characterized by unpleasant sensations and an irresistible urge to move the lower limbs. A reduction in duration of the cortical silent period (CSP) induced by transcranial magnetic stimulation, believed to reflect activity of GABAB neurons, has been inconsistently reported in RLS. Objectives: 1) To compare the CSP duration, in patients with primary RLS and in healthy subjects; 2) To correlate these measures with severity of RLS symptoms. Patients and Methods: Patients were grouped according to scores in the international RLS severity scale, IRLSS: (light/moderate [IRLSS<20] = RLSLM ;

severe/very severe [IRLSS≥20] = RLSSVS). Resting (rMT) and active (aMT) motor thresholds as well as CSP were measured. We obtained patient and Institutional Review Board (IRB) approval. Mann-Whitney tests were used to compare results in patients and in controls, and to compare results in patients with RLSLM and RLSSVS. Results: Thirty one (25F/6M) patients with primary RLS and 13 healthy subjects were included. Twenty one patients had IRLSS≥20 (RLSSVS) and 10, IRLSS<20 (RLSLM). TMS results were (RLS x controls): rMT (69.2±13.8% x 67.9 ± 13.2%; p=0.78); aMT (54.6±10.4% x 55.3±12.6%; p=0.88); CSP duration (108.7±36.3 milliseconds(ms) x 106.1±36.5 ms; p=0.83). CSP duration was not significantly different between the two groups of patients with different severities of symptoms (RLSLM 112.3±34.7 x RLSSVS 103.0±37.9; p=0.51). Conclusion: These results do not support the hypothesis that GABAB neurons of the primary motor cortex are relevant to RLS pathogenesis.

Disclosures: **A. Conforto:** A. Employment/Salary (full or part-time):; Hospital Israelita Albert Einstein, Hospital das Clinicas/Sao Paulo University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Hospital Israelita Albert Einstein, Fundação de Amparo à Pesquisa do Estado de São Paulo, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Consultant, Pfizer/BMS. **G. do Prado:** A. Employment/Salary (full or part-time):; 2.Universidade Federal de São Paulo. **E. Amaro Jr.:** A. Employment/Salary (full or part-time):; Hospital Israelita Albert Einstein, Universidade de São Paulo. **A. Eckeli:** A. Employment/Salary (full or part-time):; Universidade de São Paulo - Ribeirão Preto. **S. Magalhães:** A. Employment/Salary (full or part-time):; Hospital Israelita Albert Einstein.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.22/E3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Spontaneously emerging Parkinsonism-cerebellar syndrome in a subspecies of japanese macaque (macaca fuscata yakui): a potential analogue of multiple system atrophy

Authors: *K. W. MCCAIRN[1,2], Y. NAGAI[3], K. KIMURA[2], Y. GO[4], K. INOUE[2], M. ISODA[5], T. MINAMIMOTO[3], M. MATSUMOTO[6], T. NINOMIYA[1,2], M. TAKADA[2];
[1]Korea Brain Res. Inst., Daegu, Korea, Republic of; [2]Systems Neurosci. Section, Primate Res. Inst. - Kyoto Univ., Inuyama, Japan; [3]Mol. Neuroimaging, Natl. Inst. of Radiological Sci., Chiba, Japan; [4]Natl. Inst. of Physiological Sci., Okazaki, Japan; [5]Dept. of Physiol., Kansai Med. Univ. Sch. of Med., Osaka, Japan; [6]Div. of Biomed. Sci., Fac. of Medicine, Univ. of Tsukuba, Tsukuba, Japan

Abstract: We identified an aged Japanese macaque (Macaca fuscata yakui; 17 years old) in the Primate Research Institute colony- Kyoto University, who exhibited both parkinsonian (mild to moderate hypokinesia with stooped posture) and cerebellar signs (typically action tremor in all the axial limbs and gait instability). We used a number of investigative methodologies to classify symptom types and define the disease, including a monkey parkinsonism rating scale, EMG- and accelerometer-based classification of movement, simultaneous cortico-basal ganglia-cerebellar local field potential (LFP) recordings, genetic profiling, structural imaging using a high-intensity MRI (7 Tesla), and high resolution PET measurements of α-synuclein and dopamine transporter (DAT) levels. The LFP recording showed increased power in the high beta-frequency range ~ 20-30 Hz, relative to normal control and MPTP treated animals. Whole genome analysis of this monkey and extensive population genomic analysis of 100 macaque monkeys revealed that only this monkey possessed a deleterious homozygous deletion in SHC2 gene, a candidate gene of human multiple system atrophy (MSA). High-intensity MRI imaging showed a potential cruciform signal, like a 'hot cross bun sign', in the pons. Compared with a control subject, the α-synuclein level tended to be increased in the brainstem and cerebellum, while the DAT binding level appeared to be unaffected. In light of the above symptom profiles, we suggest that the animal may suffer from MSA, and provides a novel platform for investigating the causes of and treatments for MSA.

Disclosures: K.W. McCairn: None. **Y. Nagai:** None. **K. Kimura:** None. **Y. Go:** None. **K. Inoue:** None. **M. Isoda:** None. **T. Minamimoto:** None. **M. Matsumoto:** None. **T. Ninomiya:** None. **M. Takada:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.23/E4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Walking performance and deep brain stimulation in Essential Tremor

Authors: *C. J. HASS[1], J. SKINNER[1], R. ROEMMICH[3], M. TERZA[2], J. ROPER[2], P. ZEILAMAN[2], M. OKUN[2];
[1]Applied Physiol. and Kinesiology, [2]Univ. of Florida, Gainesville, FL; [3]Johns Hopkins, Baltimore, MD

Abstract: Essential tremor (ET) is symptomatically characterized by an action (postural and kinetic) tremor of the upper extremities, but may also involve tremors of the head, voice, trunk, and occasionally the lower extremities. Recent literature has quantitatively detected gait impairments that have been previously reported clinically. For participants with mild ET these impairments include a decreased walking velocity, decreased step length, and increased step

width as compared to healthy aged matched controls. With disease progression gait impairment worsens. Specifically, cadence decreases and a greater percentage of the gait cycle is spent in the double support phase. Deep brain stimulation (DBS) is an effective means of mitigating medication refractory tremor but early evidence suggest that ET DBS patients could possibly develop gait and balance difficulties following surgery. The purpose of this study was to systematically evaluate the effects of DBS on gait performance. Twenty-five participants (16 Male, 67 ± 8 years old) underwent the DBS procedure where stimulating electrodes were inserted unilaterally into the VIM nucleus of the thalamus. Participants were evaluated between 6-12 months post-surgery providing time to optimize the beneficial effects of surgery on tremor. During the post-surgery evaluations, participants were tested in both the DBS turned off (Off DBS - stimulation was turned off at least one hour prior to testing) and DBS turned on (On DBS - stimulation was on for 60 minutes). Retroreflective markers were placed on the participant's bony landmarks in accordance with the Vicon Plug-in-Gait full body marker set. Kinematic performance was collected at 120Hz using a 10 camera motion capture system. Participants performed 10 overground gait trials at self-selected walking speed. Tremor was assessed using the Tremor Rating Scale (TRS). Ten gait variables were compared between the baseline, DBS-on and DBS-off conditions using a one-way repeated measures ANOVA. ON-OFF DBS evaluations were conducted 9±2 months following the baseline pre-DBS evaluation. TRS scores improved following DBS implantation and optimization ($p<0.05$). However, the statistical analyses failed to detect differences in gait speed ($p=.66$), cadence ($p=.85$), stride length ($p=.27$), stride time ($p=.47$), step length ($p=.29$), step time ($p=.83$), step width ($p=.93$), percent of the gait cycle in single support ($p=.93$), double support ($p=.93$) and swing to stance ratio ($p=.92$) across the three time points. Though these findings show across the group DBS did not negatively/positively impact gait, there were patients in the cohort with observable differences post-DBS.

Disclosures: C.J. Hass: None. **J. Skinner:** None. **R. Roemmich:** None. **M. Terza:** None. **J. Roper:** None. **P. Zeilaman:** None. **M. Okun:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); royalties from book publications.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.24/E5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Pseudogene-mediated expression dysregulation contributes to the development of neurodegeneration in a haplotype-specific manner

Authors: *A. E. FURTERER, P. NGUYEN, J. CHEN, M. PRIBADI, F. GAO, M. RAMOS, G. COPPOLA;
UCLA, Los Angeles, CA

Abstract: Tauopathies are a group of neurodegenerative diseases characterized by abnormal aggregation of tau, an abundant microtubule-associated protein, and include Progressive Supranuclear Palsy (PSP), corticobasal degeneration, and forms of frontotemporal dementia and Alzheimer's disease. Rare, causal mutations within the *MAPT* gene that encodes the protein tau have been identified in families. Genetic studies have consistently linked the 17q21.31 region, containing *MAPT*, to neurodegenerative tauopathies, even when causative mutations in *MAPT* cannot be identified. The H1 haplotype at the 17q21.31 locus is the greatest risk factor for PSP and possibly other tauopathies. While the H1 haplotype occurs in ~80% of the healthy controls (the remaining 20% being H2), an estimated 97.1% of PSP patients are H1 carriers [1]. Our group previously reported methylation differences between H1 and H2 individuals, with differentially methylated probes clustering in the 17q21.31 region [2]. Analysis of microarray data from healthy controls indicates specific gene expression differences between H1 and H2 individuals. The most consistent expression change was *MAPK8IP1*, a gene located on chromosome 11. Importantly, homology analysis revealed that the microarray probe for *MAPK8IP1* is cross-reacting with LOC644172, a pseudogene within the 17q21.31 region. Though once considered defunct, certain classes of pseudogenes are now understood to perform important regulatory functions, and have been implicated in complex diseases like cancer and neurodegeneration [3,4]. Pseudogenes who share microRNA binding sites with their parent genes can act as "decoys" that compete with transcripts of the parent gene [and potentially other genes] to generate a subtle microRNA knockdown effect, or alternatively form long double-stranded RNAs that interfere with RNA stability [4]. We investigate how haplotype-associated differential expression of LOC644172 mediates neurodegenerative risk via an interaction between LOC644172 and its parent gene, *MAPK8IP1*, by overexpressing lentiviral vectors containing the sense and antisense forms of LOC644172 in human cell lines as well as induced pluripotent stem cell (iPSC) derived-neurons from patients and healthy controls. We demonstrate that pseudogene overexpression modulates endogenous *MAPK8IP1* expression at the transcriptional and translational levels using RT-qPCR and western blotting, and does so by competing for microRNA binding.

Disclosures: A.E. Furterer: None. **P. Nguyen:** None. **J. Chen:** None. **M. Pribadi:** None. **F. Gao:** None. **M. Ramos:** None. **G. Coppola:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.25/E6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: 1RO1NS056314

Title: Post-developmental LIS1 knockout in mice produces a lethal phenotype

Authors: *T. J. HINES[1], X. GAO[2], D. SMITH[2];
[1]Biol. Sci., Univ. of South Carolina, West Columbia, SC; [2]Biol. Sci., Univ. of South Carolina, Columbia, SC

Abstract: LIS1 haploinsufficiency causes lissencephaly in humans. Characteristics of lissencephaly are a smooth cortex, enlarged ventricles, cognitive deficits, and increasingly severe seizures. LIS1 is critical for proper cortical development by regulating the microtubule motor, cytoplasmic dynein. Interestingly, LIS1 expression remains high in adult nervous tissue. LIS1 and its binding partner, NDEL1, are important for axonal transport in cultured adult rat sensory neurons. In order to explore the role of LIS1 in adult animals, we are utilizing an actin-driven, tamoxifen-inducible cre recombinase mouse strain crossed to a homozygous floxed LIS1 strain to knock out LIS1 post-developmentally. The mice also express a cre reporter gene that switches from red to green fluorescence when cre becomes active. After tamoxifen injection, the cre reporter is most prominent in brainstem neurons, heart, and skeletal muscle. Cre activity is also observed in scattered astrocytes and cerebellar glia. Mosaic cre activity is observed in other tissues, such as liver, lungs, and kidneys. Brainstem neurons display signs of chromatolysis, which is a symptom of axonal damage. Dorsal root ganglion neurons cultured from these mice have more axonal swellings than controls and show defects in retrograde transport. After LIS1 knockout, the mice become very lethargic, exhibit hind leg clasping, and die within five days of tamoxifen injection. Control animals, which include vehicle-only injections or tamoxifen injected animals with either no cre or no floxed LIS1 alleles, show no observable phenotypes. While these results demonstrate the importance of Lis1 in adult animals, the precise cause of death is unknown. We have ruled out a vital role for Lis1 in cardiomyocytes and are investigating potential sources of declining health using urinalysis, electrocardiogram, and monitoring blood glucose levels. Our current model is that defective axon transport in brainstem cardiorespiratory neurons leads to widespread failure of autonomic systems.

Disclosures: T.J. Hines: None. **X. Gao:** None. **D. Smith:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.26/E7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Merz Pharma Grant # R.14.022

Title: Function improved in essential tremor by incobotulinumtoxinA injection patterns using upper limb biomechanical characterization

Authors: *O. SAMOTUS[1,3], H. V. MORADI[2], J. LEE[2], M. JOG[2];
[2]Clin. Neurolog. Sci., [1]London Hlth. Sci. Ctr., London, ON, Canada; [3]Clin. Neurolog. Sci., Western Univ., London, ON, Canada

Abstract: Background: Essential tremor (ET) is the most prevalent movement disorder and causes a loss of function in the upper extremities (UE). Oral medications are usually ineffective with frequent adverse effects which detriments a patient's quality of life. In the past, focal therapy reduced postural tremor severity but has not provided any functional benefit. Wearable motion sensors can objectively distinguish complex movements of upper limb tremor which can be utilized to accurately target muscles which generate tremor. Objective: To investigate the use of multi-sensor kinematic measures of UE tremor for selection of injection parameters, optimization and monitoring the effect of incobotulinumtoxinA for alleviating functional disability caused by essential tremor. Methods: 24 ET participants attended a total of 13 visits and were injected in UE every 16 weeks, totalling six injection cycles, and attended a follow-up visit six weeks following treatment. Clinical rating scales (item 20, 21 UPDRS, FTM, and QUEST) and kinematic assessments were completed at each visit. Participants in a seated position performed two postural and two weight-bearing functional tasks. Goniometers were placed over each UE joint to quantify tremor severity, as RMS angular amplitude, into directional components: flexion-extension (F/E), pronation-supination, and radial-ulnar in wrist, F/E in elbow, and abduction-adduction and F/E deviations in shoulder. Dosing and muscle selection were determined using kinematic data and clinician's experience. Results: Action tremor score (UPDRS item 21) showed a significant drop of 68.0% after 96 weeks. FTM tremor severity displayed a similar significant reduction of rest, postural, and action tremor by 41.7%, 80.6%, and 57.6%, respectively, at week 96. Wrist postural and action tremor total RMS amplitude significantly decreased by 86.4%. Handwriting and functional performance significantly improved by 38.3% and 41.7%, respectively. Furthermore, quality of life (QUEST) significantly improved by 43.3%. Mild weakness was experienced by less than 10% of participants and did not affect arm function. Conclusion: Kinematic assessment of tremor allows clinicians to characterize multi-joint tremor for optimal selection of injection parameters.

Disclosures: O. Samotus: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; MITACS grant in collaboration with Merz Pharma. **H.V. Moradi:** None. **J. Lee:** None. **M. Jog:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Merz Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending PCT/CA2013/000804 to MDDT Inc. and patent PCT/CA2014/050893 pending to MDDT inc. F. Consulting Fees (e.g., advisory boards); Allergan Inc, Teva Pharma, AbbVie.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.27/E8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CNPQ grant number 467566/2014-3

INNT FAPESP grant number 08/57904-0

Title: Genetic of prion diseases in Brazil

Authors: *M. C. LANDEMBERGER[1], C. F. MACHADO[2], J. SMID[3], H. R. GOMES[3], L. CHIMELLI[4], N. H. S. CANEDO[4], S. ROSEMBERG[3], R. NITRINI[3], V. R. MARTINS[1]; [1]Mol. and Cell Biol., A.C. Camargo Cancer Ctr., São Paulo, Brazil; [2]IQ, [3]USP, São Paulo, Brazil; [4]UFRJ, Rio de Janeiro, Brazil

Abstract: Global surveillance of vCJD and other forms of CJD was recommended from the WHO for a better understanding of potential causes of iatrogenic CJD, as well as the distribution of various hereditary forms. Prion diseases have been under compulsory notification in Brazil since 2005. From 2005 to 2015, we have received 434 blood samples from notified cases of suspected CJD. Blood samples were analyzed by direct genomic sequencing to identify mutations and polymorphisms in the PRNP gene. Cases with mutation in direct sequencing were cloned to confirm results. The presence of 14.3.3 protein in Cerebrospinal Fluid (CSF) was evaluated using immunoblotting and brain tissue obtained by autopsy or biopsy was analyzed by imunohistochemistry for the presence of spongiosis and proteinase K resistant PrP. The average age of our patients was 60.3 years (range 10-94 years), males representing 52% of the cases. PRNP polymorphisms analysis showed that 51% of the cases were homozygous for methionine at codon 129 (M129M), 30% were heterozygous (M129V) and 19% were homozygous for valine (V129V). The silent polymorphism at codon 117 was detected in 10% of the patients and 5% had deletion at the octarepeat. . Regarding genetic diseases, we found fifteen patients with CJD, in which the mutation E200K (nine cases), D178N (two cases), T183A (one case), V180I (one case) and octarepeat insertion (two cases) were detected. We also diagnosed two patients with GSS syndrome (P102L) and three patients with fatal familial insomnia (129M+178N). Brain tissue of 32 patients was available, 28 (87%) of them had spongiosis and were positive for proteinase K resistant PrP. After clinical evaluation, imaging exams, 14.3.3 protein presence, genetic and immunohistochemical analysis, the notified cases were classified according to the WHO criteria. Among of them, 8.3% were classified as sporadic CJD, 39% as probable CJD, 18% as possible CJD, 4.6% as genetic prion disease, 23.6% as suspected CJD and 6.5% were non-CJD. This study provides the first epidemiologic data about human prion diseases in Brazil. Similar to any other country the availability of brain tissue from these patients is a limiting factor to confirm the diagnosis of prion diseases. This study also represents an important tool for prion-prevention policies and is of great importance for future implementation of clinical trials.

Disclosures: **M.C. Landemberger:** None. **C.F. Machado:** None. **J. Smid:** None. **H.R. Gomes:** None. **L. Chimelli:** None. **N.H.S. Canedo:** None. **S. Rosemberg:** None. **R. Nitrini:** None. **V.R. Martins:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.28/E9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Microglia and the complement pathway in sandhoff and tay-sachs disease

Authors: *B. JUN, F. S. EICHLER;
Neurol., MGH, Boston, MA

Abstract: Sandhoff (SD) and Tay-Sachs Disease (TSD), caused by dysfunctional subunits of the β-hexosaminidase A and B enzymes, are characterized by excessive accumulation of GM2 gangliosides in neurons. In patients and $HexB^{-/-}$ mouse models, GM2 storage leads to synaptic and neuronal loss, leading to a devastating neurological phenotype (Myerowitz et al., 2002). In addition, neuropathology is accompanied by widespread neuroinflammation indicated by microglial activation. However, whether the inflammatory contributions are primary or secondary to the neurodegeneration remains unknown. Recent RNA sequencing data revealed that the *HexB* gene is exclusively expressed in microglia, the immune cells of the CNS, suggesting that the gene defect may be affecting the neuro-immune response (Hickman et al., 2013). Interestingly, the Complement immune pathway, which mediates synaptic regulation during neuronal pruning, has been implicated in neurodegenerative diseases such as AD (Stephan et al., 2012). Here, we report that in $HexB^{-/-}$ mice, but not in WT and $HexB^{+/-}$, there is a marked increase in Complement C1q expression in the cerebellar, cortical, and subcortical brain regions. C1q was localized to neurons and their processes, suggesting opsonization and elimination of these targets. The increase of C1q was confirmed in post-mortem samples of a TSD patient cortex. Further, we found that $HexB^{-/-}$ microglia display elevated expression of Complement receptor 3 (CR3/CD11b), suggesting that they are primed to respond via the classical Complement cascade. Our data support the role of the Complement pathway as a mechanism of neuronal loss induced by activated microglia in GM2 gangliosidoses. **References**
Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang L, Means TK, El Khoury J. (2013) The microglial sensone revealed by direct RNA sequencing. *Nat Neurosci*. 16(12):1896-1905. Myerowitz R, Lawson D, Mizukami H, Mi Y, Tifft CJ, Proia RL. (2002) Molecular pathophysiology in Tay-Sachs and Sandhoff diseases as revealed by gene expression profiling. *Hum Mol Gen*. 11(11):1343-1350. Stephan AH, Barres BA, Stevens B. (2012) The Complement

System: An Unexpected Role in Synaptic Pruning During Development and Disease. *Annu Rev Neurosci*. 35:369-389.

Disclosures: B. Jun: None. **F.S. Eichler:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.29/E10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH (R01GM084979, 3R01GM084979-02S1)

Title: Propofol induces autophagy by causing calcium release from endoplasmic reticulum via activation of IP_3R

Authors: G. REN, *M. F. ECKENHOFF, H. WEI;
Anesthesiol. & Critical Care, Perelman Schl Med. UPENN, Philadelphia, PA

Abstract: Our previous studies have demonstrated that inhalational anesthetic isoflurane abnormally raises cytosolic calcium levels and causes cell death by apoptosis. We hypothesize here that intravenous anesthetic propofol affect the autophagy process by causing calcium release from the endoplasmic reticulum via inositol 1,4,5-trisphosphate ($InsP_3R$) receptors, which is associated its effect on cell survival. For these studies, chicken B lymphocytes with all three $InsP_3R$ isoforms knocked out (DT40-TKO) or with only the type I receptor (DT40-R1), and SH-SY5Y human neuroblastoma cells were treated with propofol at 200 and 250 μM. Cytosolic calcium concentrations ($[Ca^{2+}]_c$) were measured using Fura-2 340/380 ratio and cell viability was determined by LDH and MTT assays. Autophagy activity was monitored by measuring levels of LC3-II using Western blot assays. We found that a short treatment (2.5 hours) with 200 μM propofol induced autophagy in SH-SY5Y cells, which protected these cells from propofol's cytotoxicity. Propofol obviously increased $[Ca^{2+}]_c$. Chelation of cytosolic calcium with BAPTA-AM decreased LC3-II levels induced by propofol and resulted in increased cell death, suggesting an adequate baseline level of $[Ca^{2+}]_c$ is required for adequate autophagy function and maintenance of cell survival. Furthermore, propofol induced autophagy only in the DT40-R1 but not in the DT40-TKO cells, suggesting type 1 $InsP_3R$ is required for propofol to regulate autophagy. Inhibition of calcium release from $InsP_3R$ in SH-SY5Y cells with Xestospongin C also reduced autophagy activity induced by propofol. In summary, a short exposure to propofol induces autophagy which requires adequate calcium release from the endoplasmic reticulum via $InsP_3R$ and provides cytoprotection.

Disclosures: G. ren: None. **M.F. Eckenhoff:** None. **H. wei:** None.

Poster

304. Frontotemporal Dementia and Other Neurodegenerative Disease

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 304.30/E11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH/NINDS 1R01NS086810

Michigan Protein Folding Disease Initiative - Pilot Drug Screen

Title: High-throughput screen for inhibitors of RAN translation

Authors: *K. M. GREEN[1], M. G. KEARSE[2], P. K. TODD[2,3];
[2]Neurol., [1]Univ. of Michigan, Ann Arbor, MI; [3]Veteran Admin. Med. Ctr., Ann Arbor, MI

Abstract: Repeat associated non-AUG (RAN) translation is a non-canonical translational initiation event that occurs at nucleotide repeat expansions associated with neurodegenerative diseases such as ALS, frontotemporal dementia, and ataxia. Translation through expanded repeats results in production of toxic homopolymeric or dipeptide repeat-containing proteins that accumulate in the brains of patients with repeat expansion disorders and contribute to disease pathogenesis. Consequently, RAN translation constitutes a potentially powerful therapeutic target for multiple neurodegenerative disorders. We therefore developed an assay to screen for small molecule inhibitors of RAN translation associated with CGG repeat expansions at the fragile X locus. Such expansions cause Fragile X-associated Tremor Ataxia Syndrome and trigger RAN translation of a toxic polyglycine protein, FMRpolyG, in patients and animal models. This assay utilizes a RAN translation reporter with 100 CGG repeats placed upstream of a modified luciferase. *In vitro* translation of this reporter RNA in a rabbit reticulocyte lysate yields a robust and quantitative signal that is specific to the RAN initiation product. Using this system, we screened 3254 bioactive compounds. We obtained an average Z score per plate of 0.79 and an average well-to-well variation (CV) of 6.93%. We identified 211 compounds that reduced signal by at least 3 standard deviations relative to controls. Several classes of compounds were highly represented within this group, including known protein synthesis inhibitors, DNA/RNA intercalators, and free radical scavengers. We are now performing counter screens and dose response assays to identify compounds that selectively inhibit RAN translation and these will be advanced to *in vivo* disease models. This approach should be applicable to other repeat expansions and could serve as a template for therapeutic development in neurodegenerative disorders.

Disclosures: K.M. Green: None. **M.G. Kearse:** None. **P.K. Todd:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.01/E12

Topic: C.05. Aging

Support: NIH grant AG044919

Title: Disrupted fractalkine signaling impairs synaptic plasticity and long term memory retention

Authors: B. GRIMMIG[1], L. DESFOSSES[2], L. DALY[2], C. HUDSON[3], X. WANG[4], E. WEEBER[4], *P. C. BICKFORD[2,3];
[1]Neurosurg. and Brain Repair, USF, Tampa, FL; [2]Neurosurg. and Brain Repair, USF Morsani Col. of Med., Tampa, FL; [3]Res. Service, James A Haley Veterans Hopsital, Tampa, FL; [4]USF Byrd Alzeimers Ctr., Tampa, FL

Abstract: CX3CL1 (Fractalkine) is a chemokine that regulates microglial activity and has been shown to protect the brain from the damaging consequences of chronic inflammation. Microglia have also been implicated as critically involved in sculpting neural circuits in the developing CNS as well as refining those in adulthood during periods of learning. Both the expression of CX3CL1 and microglial function are affected by age, possibly compromising the capacity for synaptic remodeling thus negatively impacting neural plasticity. Here, we characterize the cognitive behavioral effects of disrupted microglial activity in fractalkine knock out mice at different ages. CX3CL1-/- mice show impaired recall in a contextual fear conditioning task beginning at 14 days post training at 3 months of age and this deficit remains at 15 months of age. Although these mice do not show abnormal locomotor activity they do show superior motor learning at 3 months of age, however this difference is not maintained as they age. Impaired neurogenesis was also observed, indicated by a reduced number of both Ki67 and doublecortin labeled cells in the subgranular zone of the hippocampus at 3 months of age. However this deficit is not observed at later ages. We also examined synaptic function in CX3CL1-/- knock out mice and found age-dependent alterations in synaptic plasticity for both NMDA receptor-independent and -dependent long-term potentiation.

Disclosures: B. Grimmig: None. **L. DesFosses:** None. **L. Daly:** None. **C. Hudson:** None. **X. Wang:** None. **E. Weeber:** None. **P.C. Bickford:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.02/E13

Topic: C.05. Aging

Support: NIH Grant NS R01084817

NIH Grant DA R01033966

NIH Grant NS R01060632

Title: Chronic HIV exposure and aging render mPFC pyramidal neurons in the reward circuits more vulnerable to excitatory stimuli

Authors: *L. CHEN[1,2], C. E. KHODR[1,2], S. DAVE[1,2], L. AL-HARTHI[3], X.-T. HU[1,2];
[1]Dept. of Pharmacol., [2]Ctr. for Compulsive Behavior & Addiction, [3]Dept. of Immunol. and Microbiology, Rush Univ. Med. Ctr., Chicago, IL

Abstract: The medial prefrontal cortex (mPFC) plays an important role in regulating cognitive function and reward-associated behaviors, and is dysregulated in HIV+ humans. Despite combined antiretroviral therapy (cART), neurological and neuropsychiatric deficits are still prevalent among HIV infected patients. Therefore, it is important to understand the neuropathophysiology in the mPFC associated with HIV neuroinvasion. Our previous studies showed that acute exposure to HIV-1 protein Tat induces hyper-excitation of mPFC pyramidal neurons in adolescent (~5-6 week of age) and young adult (5-6 month of age) rats, indicating that prolonged exposure to HIV-1 Tat cause neuronal injury/death. Our recent studies using HIV-1 transgenic (Tg) rats, which express 7 of the 9 HIV-1 proteins (env, tat, rev, nef, vpr, vif, and vpu), further reveal that mPFC pyramidal neurons in adolescent HIV-1 Tg rats also exhibit hyper-excitability. To determine the influence of aging and effects chronic exposure to low levels of HIV-1 proteins *in vivo*, we evaluated the neuropathophysiological properties of mPFC pyramidal neurons from 12-month-old HIV-1 Tg rats and compared that to those from age-matched non-Tg rats. Whole-cell patch-clamp recordings were performed for such evaluation. We found that mPFC pyramidal neurons from aged HIV-1 Tg rats showed a significant increase in evoked firing as compared to those from age-matched non-Tg rats. Such increased firing in neurons from aged HIV-1 Tg rats was significantly reduced by selective blockade of L-type Ca2+ channels (L-channel) with nifedipine (5 μM, acutely applied in bath), indicating involvement of the L-channels in this neuronal hyper-excitation. Additionally, we also found significant changes in the membrane properties (e.g., more depolarized resting membrane potential and decreased rheobase, a minimal depolarizing current that was required for generation of firing), which confirmed increased excitability of mPFC pyramidal neurons in aged HIV-1 Tg rats. These novel findings reveal that, like in the younger rats, the mPFC neuronal function in aged HIV-1 Tg rats is also altered following chronic exposure to HIV-1 proteins *in vivo*. Combined chronic HIV exposure and aging render mPFC pyramidal neurons in the reward circuits more vulnerable to excitatory stimuli. Whether and to what extent such mPFC neuropathophysiology found in the neurons from aged HIV-1 Tg rats was greater than that seen in the neurons from adolescent rats are currently under investigation.

Disclosures: **L. Chen:** None. **C.E. Khodr:** None. **S. Dave:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.03/E14

Topic: C.05. Aging

Support: NIA Grant 5T32AG000057-38

NIH grant NS076416

UW RRF grant

Intramural Research Program of the NIH, NIA

Title: Increased risk aversion with age on a probability discounting task

Authors: *V. L. TRYON[1], H. O. KING[1], J. M. LONG[2], P. R. RAPP[2], S. J. Y. MIZUMORI[1]; [1]Psychology, Univ. of Washington, Seattle, WA; [2]Lab. of Behavioral Neurosci. at the Natl. Inst. on Aging, NIH, Baltimore, MD

Abstract: Normal cognitive aging results in changes in risk-based decision making. The goals of the current study were to characterize how aging may influence risk-based decision making on a probability discounting task using rats, and then test whether a mitochondria enhancing drug impacts the age-related effect. The task tested rats' preference for a lever that lead to a large reward with varying probability (the risky option) or a lever that lead to a small reward 100% of the time (the certain option). Pressing the certain lever led to the delivery of 1 sugar pellet 100% of the time while pressing the risky lever led to 4 sugar pellets with varying probability across trial blocks. The descending probabilities associated with the risky lever were 100%, 50%, 25%, and 12.5%. A session consisted of 8 blocks of trials, a forced choice block followed by a free choice block. Each forced choice block contained 8 trials in which one lever was presented at a time to inform the rat of the current reward probability. Next were 10 free choice trials in which both levers were made available, and rats were free to choose amongst the 2 options. Then, we tested the impact of a therapeutic intervention using a mitochondria targeted drug, SS-20 on the deficits observed in aged animals' decision making strategies. SS-20 is a cardiolipin-targeted agent that has been shown to enhance mitochondrial energetics. As expected, based on earlier studies with aged animals, the aged rats displayed significantly increased discounting behavior compared to young controls. Interestingly, the degree of probabilistic discounting was correlated with performance on a spatial memory test using the Morris water maze. However, at this time, it does not appear that SS-20 changed the discounting behavior in young or aged animals. It is possible that SS-20 is improving the energetics of neural tissue but that the test was not sensitive enough to pick up these effects. Indeed, open field tests that were also administered before and after drug exposure revealed that the peptide may have a protective effect on age-related physical

deterioration. To investigate how reward processing may change in the aged brain, an additional study is underway in which neural activity from midbrain regions important for the processing of rewards is being recorded in another group of young and aged animals during a different version of the probabilistic discounting task. Our findings should help explain why older animals are more risk averse than their younger counterparts. Together, this work provides further details of altered decision making strategies with age and seeks to elucidate possible neural mechanisms that may underlie their altered behavior.

Disclosures: **V.L. Tryon:** None. **H.O. King:** None. **J.M. Long:** None. **P.R. Rapp:** None. **S.J.Y. Mizumori:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.04/E15

Topic: C.05. Aging

Support: NIH Grant R01AG043467

Title: Examination of sex differences in c-Fos expression in adult and aged Fischer 344 rats reveals that females, but not males, display enhanced neural activation after social interaction

Authors: *A. E. PERKINS[1], R. L. SPENCER[2], E. I. VARLINSKAYA[1], E. A. WOODRUFF[2], L. A. CHUN[2], J. M. INTERRANT[1], T. DEAK[1];
[1]Binghamton Univ., Binghamton, NY; [2]Univ. of Colorado-Boulder, Boulder, CO

Abstract: There is a significant decline in social behavior across the lifespan. This can be detrimental to aging individuals, since positive social interaction produces significant health benefits. Neural activation patterns following social experience in rodents may provide insight into factors that mediate an aging-related decline in social behavior. To answer this question, two experiments were conducted in parallel. In Exp. 1, 3-4 month-old adult male and female F344 rats (n = 6-8/group) were left undisturbed in their home cage as controls (HCC), exposed to the testing context alone for 30 min (CXT), or exposed to the context for 20 min, followed by a 10 min social interaction (SI) test with an age- and sex-matched conspecific. C-Fos induction was assessed by *in situ* hybridization in hippocampus (CA1, CA3, & DG), barrel cortex (BF layers 2/3, 4, & 5/6), cingulate cortex (CING layers 2/3, 4, & 5/6), medial amygdala (MeA), and the paraventricular nucleus of the hypothalamus (PVN). In the second experiment, the same behavioral testing parameters were used in 3- and 18-month-old male F344 rats (n = 6-9/group),with expression of c-Fos, cytokines (IL-6, IL-1, & TNFα), and social peptide receptors (OTR & AVPR1a) assessed in the PVN and amgydala (AMG) using RT-PCR. In the first experiment, analysis of social behavior revealed that females displayed more social investigation

than males. Additionally, in DG, females demonstrated greater c-Fos induction after social interaction (relative to being in the context alone), whereas males displayed identical increases in c-Fos when exposed to context alone or a social partner. When cytokine expression was examined in male 3- and 18-month old rats in Exp. 2, IL-6 was significantly elevated in the AMG following either CXT or SI, regardless of age. Exposure to CXT or SI did not influence expression of OTR or AVP1a in either the AMG or PVN. Notably, however, a similar pattern in c-Fos induction was observed in the AMG of males via both measurement approaches (*in situ* hybridization and RT-PCR). Taken together, these data confirm the use of this behavioral paradigm to explore aging-related changes in the circuitry that underlies the response to social interaction. Overall, marginal increases in c-Fos in the PVN suggest that the social testing procedure is minimally stressful, yet the profound induction in other structures confirms the sensitivity of this model for studying the neural circuitry of social behavior. Given the profound sex differences observed in Exp. 1, future studies will extend this research into aged females while continuing to explore the involvement of immune factors and social peptides in aging-related changes in social behavior.

Disclosures: **A.E. Perkins:** None. **R.L. Spencer:** None. **E.I. Varlinskaya:** None. **E.A. Woodruff:** None. **L.A. Chun:** None. **J.M. Interrant:** None. **T. Deak:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.05/E16

Topic: C.05. Aging

Support: Research Foundation of Korea(2012M3A9C6049937)

Research Foundation of Korea(2011-0019354)

Title: Changed HRG expression and cell types in micro-environment of old NSC niche

Authors: Y. KIM[1], H. LEE[2], Y. JUNG[2], *Y. K. KWON[1];
[1]Kyung Hee Univ., Seoul,, Korea, Republic of; [2]Life and Nanopharmaceutical science, Kyunghee university, Seoul, Korea, Republic of

Abstract: Heregulin(HRG) is a component of neural stem cell (NSC) niche regulating self-renewal and differentiation in NSC microenvironment. NSCs of old mice brain are slow in self-renewal and their developmental potential is reduced. NSC niche in old mice brain may be different from young mice. Here we compared the expression of HRG and its receptor, ErbB4, and expression of their target genes in NSC niche of young and old mice brain. First, we searched what cell types in brain are changed between young and old mice using transgenic mice expressing pax6-GFP. We found Pax6+GFP expressing NSCs were remarkably reduced in

addition to proliferating type2 cells. ErbB4+type1 cells and DCX+type3 cells were also diminished. When we isolated NSC from SVZ, the size and numbers of neurospheres cultured from older brain were smaller and expression of NeuN, GFAP, vGluT1 and GAD in neurospheres from older brain were diminished in immunoblot assays. Expression of HRG-β mRNA together with DISC-1 and Dlx2 in neurospheres cultured from SVZ of the older brains also decreased but HRG-α increased slightly in RT-PCR assay. In young brains NRG-EGF domains and NRG-ICD are expressed in brain vascular pericytes but not in the older brains. Numbers of brain vascular pericytes and expression of ErbB4 are diminished in older brains. This suggests reduced expression of HRG may be a cause of changed micro-environment of old NSC niche. This work was supported by grants from National Research Foundation of Korea(2012M3A9C6049937)and (2011-0019354)

Disclosures: **Y. Kim:** None. **H. Lee:** None. **Y. Jung:** None. **Y.K. Kwon:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.06/E17

Topic: C.05. Aging

Support: NIH Grant 5P01AG026572 to RDB; Project 1 to RDB & EC.

Title: Aging transition of perimenopause is associated with bioenergetic shift and decline in long-term potentiation in the female brain

Authors: ***F. YIN**[1], J. YAO[1], H. SANCHETI[1], T. FENG[1], T. E. MORGAN[2], C. E. FINCH[2], C. J. PIKE[2], W. J. MACK[3], E. CADENAS[1], R. D. BRINTON[1];
[1]Sch. of Pharm., [2]Davis Sch. of Gerontology, [3]Keck Sch. of Med., USC, Los Angeles, CA

Abstract: Perimenopause is a transition state of female aging that proceeds- and leads to reproductive senescence and is associated with multiple neurological symptoms, including those associated with increased Alzheimer's disease (AD) risk, such as depression, insomnia and subjective memory impairment. The current study determined the biological transformations that occur in the aging female brain during the perimenopausal transition and its implications for AD risk. A preclinical model of human perimenopause was developed that assessed chronological and endocrine aging in female rats. Levels of key neuroactive steroids in serum and brain were determined by LC-MS/MS. Bioenergetic-, redox-, inflammatory-, and AD pathology-related gene expression was determined by customized low density gene array followed by bioinformatic analysis. The perimenopausal transition state was further characterized by the expression of key metabolic enzymes, the activity of signaling pathways, and mitochondrial respiratory capacity. Functional outcomes were assessed in this model using (a) FDG-microPET

for cerebral glucose metabolism, (b) long-term potentiation for synaptic plasticity, and (c) glucose tolerance test for peripheral metabolic status. Gene expression analyses indicated two distinct aging programs: chronological and endocrine. Modest decline in bioenergetic gene expression occurred with chronological aging. Conversely, a critical period emerged during the endocrine transition from regular to irregular cycling characterized by a down-regulation of genes required for glucose metabolism and mitochondrial function, which were confirmed by declines in brain glucose metabolism, mitochondrial capacity and synaptic plasticity. Bioinformatic and biochemical analyses indicated that bioenergetic profiles were likely regulated by the upstream insulin/IGF1 and AMPK/PGC1α signaling pathways. The onset of acyclicity was accompanied by a rise in genes required for mitochondrial function, inflammation, and fatty acid metabolism. Subsequent chronological aging resulted in an overall decline in genes involved in mitochondrial function and β-amyloid degradation. These findings provide novel mechanistic insights into the impact of the aging perimenopausal transition on brain metabolism and synaptic function, which could have implications for identifying phenotypes of AD risk and a critical window for earliest detection in aging females. This work was supported by NIA 5P01AG026572 to RDB; Project 1 to RDB & EC.

Disclosures: F. Yin: None. **J. Yao:** None. **H. Sancheti:** None. **T. Feng:** None. **T.E. Morgan:** None. **C.E. Finch:** None. **C.J. Pike:** None. **W.J. Mack:** None. **E. Cadenas:** None. **R.D. Brinton:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.07/E18

Topic: C.05. Aging

Support: NIH Grant 5P01AG026572 to RDB; Project 1 to RDB & EC.

Title: Continuous 17β-estradiol combined with cyclic progesterone modulates female brain bioenergetic functions in an endocrine status dependent manner

Authors: *Z. MAO[1], F. YIN[1], J. YAO[1], T. E. MORGAN[2], E. CADENAS[1], R. D. BRINTON[1]; [1]Sch. of Pharm., [2]Davis Sch. of Gerontology, USC, Los Angeles, CA

Abstract: The perimenopause is an aging transition unique to the female that is associated with multiple neurological symptoms. Our recent study in a rodent model of human perimenopause revealed the perimenopausal transition from regular- to irregular cycling as a critical period for brain metabolic function, characterized by a significant decline in bioenergetic and synaptic functions. Combinations of estrogens and progestogens in varying regimens are widely used as hormone therapy for menopause-related climacteric symptoms. We previously reported that a

two-month treatment of continuous 17β-estradiol (E2) in combination with cyclic progesterone (P4) (E2+CyP4) on ovariectomized (OVX) young rats (3-month-old) induced a bioenergetic gene-expression profile comparable to the ovary intact females. The present study was aimed to determine the efficacy and optimal intervention window of the E2+CyP4 therapy on female rat brain during the perimenopausal transition. Placebo or E2+CyP4 therapy was initiated on female rats at 9-10 months old with either regular cycling or irregular cycling, and for each cycling status, Sham OVX or OVX surgery was performed before the intervention. Hormone therapy consisted of two 30-day cycles of continuous E2 and cyclic P4 (10 days/cycle) delivered by silastic capsules. Upon completion of the regime, rats were subject to genomic, biochemical and brain metabolic investigations. Our preliminary data indicate that the efficacy of E2+CyP4 therapy on brain bioenergetic functions in terms of glucose metabolism and mitochondrial respiratory capacity was differentially affected by the endocrine status of the rats when the intervention was initiated. Outcomes of this study in the model of natural perimenopause will determine the window of opportunity for preventing the at-AD-risk bioenergetic phenotype by hormone intervention and will provide mechanistic details for developing novel strategies to maintain neurological health and function throughout menopausal aging. This work was supported by NIA 5P01AG026572 to RDB; Project 1 to RDB & EC.

Disclosures: **Z. Mao:** None. **F. Yin:** None. **J. Yao:** None. **T.E. Morgan:** None. **E. Cadenas:** None. **R.D. Brinton:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.08/E19

Topic: C.05. Aging

Support: NIH Grant 5P01AG026572 to RDB; Project 5 to RDB.

Title: Mechanism of perimenopausal hot flash: involvement of brain hypometabolism and mitochondrial uncoupling

Authors: *R. D. BRINTON, F. YIN, J. YAO, A. MISHRA;
Sch. of Pharm., USC, Los Angeles, CA

Abstract: The goal of the current study is to determine the mechanism of the signature symptom of the menopausal transition, the perimenopausal hot flash. We hypothesized that loss of ovarian hormone regulation of bioenergetics in brain induces a series of adaptive responses in the brain bioenergetic system, that are initiated by decline in glucose metabolism, followed by activation of alternative fuel sources, ketones and free fatty acids, which lead to mitochondrial uncoupling and temperature dysregulation. We used the ovariectomy (OVX) rat model as a reliable and

predictive inducer of temperature dysregulation. Loss of ovarian hormones in the OVX rats led to decreased uterine weight, increased body weight and most importantly, a significant increase in peripheral (tail skin) temperature. Further, our analyses in the OVX rat model indicated that peripheral temperature dysregulation coincided with decreased cerebral glucose metabolism (FDG-PET) in brain and systemic glucose intolerance. OVX-induced changes were completely or partially prevented by 17beta-estradiol treatment, suggesting an obligatory role of estrogen signaling in these events. We further tested our hypothesis that loss of ovarian hormones leads to disruption and uncoupling of the proton motive force-dependent energy conservation systems and the consequent dissipation of energy as heat. Results of these analyses indicated that mitochondrial respiratory control ratio (RCR) was decreaed in OVX rats and was accompanied by mitochondrial uncoupling. In parallel, the uncoupling of brain mitochondria in OVX'd rats was concurrent with the upregulation of mitochondrial uncoupling proteins (UCPs) in multiple brain regions. Finally, to investigate the relationship between mitochondrial uncoupling in the brain and the increase in peripheral temperature, we induced mitochondrial uncoupling in the brain by intracerebroventricular injection of 2,4-dinitrophenol (2-DNP), a mitochondrial uncoupler. Preliminary analyses indicate that injection of 2-DNP into brain induced a rise in tail skin temperature as well as fluctuations in brain temperature. Collectively, we established the physiological and bioenergetic phenotype of a rat model of hot flash, and using this model, our findings provide new mechanistic details of hot flash by connecting loss of ovarian hormones, brain hypometabolism, mitochondrial uncoupling and dysfunction, and peripheral temperature dysregulation. This work was supported by NIA 5P01AG026572 to RDB; Project 5 to RDB

Disclosures: R.D. Brinton: None. **F. Yin:** None. **J. Yao:** None. **A. Mishra:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.09/E20

Topic: C.05. Aging

Support: NIA P01AG026572

NIA R01AG032236

Title: Mechanistic pathways linking mitochondrial hydrogen peroxide production and white matter degeneration in the aging mammalian female brain

Authors: *L. KLOSINSKI[1], J. YAO[1], S. CHEN[1], Z. MAO[1], E. TRUSHINA[2], S. TIWARI-WOODRUFF[3], L. ZHAO[4], R. BRINTON[1];
[1]USC, Los Angeles, CA; [2]Mayo Clin., Rochester, MN; [3]Univ. of California Riverside, Riverside, CA; [4]Univ. of Kansas, Lawrence, KS

Abstract: White matter hyperintensities are an early hallmark of Alzheimer's Disease (AD) which ultimately manifest in later stages of the disease as white matter degeneration. We propose a mechanistic link between white matter hyperintensities / degeneration and mitochondrial H2O2 generation which activates the phospholipase A2 (PLA2) and arachidonic acid (AA) pathway to initiate a cascade of white matter degradation in the aged mouse brain. Female mice were sacrificed at 3, 6, 9, 12, and 15 months of age followed by analyses of gene and protein expression, electron microscopy, lipidomics and immunohistochemical analysis, coupled with enzyme activity. The degenerative cascade begins with a statistically significant increase in PLA2 activation at 12 months of age. Consistent with increased PLA2 enzyme activity, myelin degradation associated genes, AA and alkaline ceramidase, were significantly upregulated at 12 months of age. Upregulation of AA epoxygenase ($p=0.004$) is indicative of an increase in AA availability, while an increase in alkaline ceramidase ($p=0.01$) indicates activation of the sphingomyelinase ceramide pathway. While myelin synthesis genes exhibited a pattern of downregulation between 12 and 15 months of age, myelin degradation genes remained upregulated, indicating that white matter degeneration continues after 12 months of age. Immunohistochemical mapping of myelin basic protein indicated that myelin area increased in major myelin tracts between 9 and 12 months, followed by a precipitous decline between 12 and 15 months. Electron microscopy analysis of myelin organization revealed that the expansion of myelin area was due to loss of myelin compactness and structural integrity. Structural changes in myelin integrity were widely manifested as a phenotype of distorted myelin sheaths. Myelin degeneration was further confirmed by lipidomic analysis that revealed an increase in myelin's constituent components, ceramides and fatty acids, at 12 and 15 months respectively. These findings create a mechanistic foundation and temporal trajectory for progression of white matter degeneration in aged brain. Research supported by NIA P01AG026572 (Project 1 to RDB) and NIA R01AG032236 to RDB.

Disclosures: **L. Klosinski:** None. **J. Yao:** None. **S. Chen:** None. **Z. Mao:** None. **E. Trushina:** None. **S. Tiwari-Woodruff:** None. **L. Zhao:** None. **R. Brinton:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.10/E21

Topic: C.05. Aging

Support: NIA 5P01AG026572 to RDB; Project 1 to RDB & EC

Title: Mitochondrial gene expression during perimenopause and chronological aging

Authors: *Y. WANG[1], F. YIN[2], R. D. BRINTON[2];
[1]Clin. and Exptl. Therapeutics, Sch. of Pharm., [2]Pharmacol. and Pharmaceut. Sciences, Sch. of Pharm., USC, Los Angeles, CA

Abstract: Human mitochondrial genome contains 37 genes, including 13 protein-encoding genes, which are all core subunits belonging to complexes I, III, IV, or V of the electron transport chain (ETC). Alterations in gene expression can lead to changes in cellular respiration and bioenergetics, which are implicated in multiple neurodegenerative diseases including Alzheimer's disease. Compared to males, females over 60 years old are at greater risk for Alzheimer's disease. To investigate the effects of endocrine transition during perimenopause and chronological aging on mitochondrial gene expression in female brain, we used a rat model recapitulating fundamental characteristics of the human perimenopause. Specifically, female Sprague-Dawley between 9-10 months old were classified as either regular cycling, irregular cycling, or acyclic based on their estrus status. 6-month-old regular cycling and 16-month-old acyclic rats were included to distinguish the effect of chronological aging from endocrine aging. We observed that in the hippocampus, MT-ND3 (complex I), MT-CYB (complex III), and MT-ATP6 (complex V) had significantly lower expression in both irregular and acyclic 9-month-old animals comparing to regular cyclic 9 month ones; MT-CO1, MT-CO2, and MT-CO3 had significantly lower expression in acyclic 9-month old animals compared to regular cyclers. Although other protein coding genes did not show significant differences, they did share a similar trend in decreased gene expression. In terms of chronological aging, relative to 6 month old female rats, 9 month old animals exhibited an increase in mitochondrial genes expression in hippocampus, whereas a decline in mitochondrial gene expression occurred by 16 months of age. In contrast, the cerebral cortex exhibited a different pattern of gene expression. During perimenopause, mitochondrial gene expression patterns in irregular cycling and acyclic rats were not significantly different from regular cyclers. Rather than have a surge in expression at 9 month as seen in hippocampus, mitochondrial gene expression in cortex continuously decreased as animals aged, and at 16-month-old, 8 out of 13 protein coding genes spanning ETC complexes I, III, IV, and V were significantly lower level compared to 6-month-old animals. Our data suggest that in the hippocampus, mitochondrial gene expression is sensitive to both endocrine and chronological aging. We have also shown regional differences in mitochondrial gene expression between the chronological and endocrine programs with the hippocampus exhibiting both chronological and endocrine aging whereas the cerebral cortex exhibited only chronological aging.

Disclosures: Y. Wang: None. **F. Yin:** None. **R.D. Brinton:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.11/E22

Topic: C.05. Aging

Support: NIA RO1AG033605 (TRP)

Title: Expression of alternative splicing factors change in a brain region-specific manner with loss of circulating 17β-estradiol in the aged female rat brain

Authors: *C. L. SHULTS, E. PINCETI, Y. S. RAO, T. R. PAK;
Cell. & Mol. Physiol., Loyola Univ. Chicago, Maywood, IL

Abstract: Aging increases alternative splicing (AS) events in the brain of both healthy and neurodegenerative individuals. Loss of circulating 17β-estradiol (E2) associated with menopause further compounds the effects of aging in women, yet increases in age-related AS have not been studied in females. Age-related changes in the expression of the CNS-specific RNA-binding splicing factor NOVA1 have been implicated in increased AS events in male-only studies. There are many important splicing-related factors, like NOVA1, present in the brain that may be affected not only by aging, but also by loss of circulating E2. We hypothesized that splicing factor expression changes with aging and longer periods of E2 deprivation in a brain region-specific manner. In our model of surgically-induced menopause, 18 month old animals were ovariectomized (OVX) , and then, after varying deprivation periods (1 wk, 4 wks, 8 wks, 12 wks), were treated with either vehicle or 2.5 ug/kg E2 for 3 consecutive days. All animals were euthanized 24 hours after the last treatment for tissue collection of the hypothalamus, dorsal hippocampus, and ventral hippocampus. Expression of three splicing factors, HNRNPH1, DDX17, and CELF5, all significantly changed in brain region-specific manner with longer periods of E2 deprivation and subsequent E2 treatment. Expression of three other splicing factors, NOVA1, CELF4, and RBFOX1, were significantly altered in a region-specific manner, but not in every brain region. For example, NOVA1 expression was significantly altered in the hypothalamus and ventral hippocampus, but not in the dorsal hippocampus. Interestingly, there was a significant interaction between length of E2 deprivation and E2 treatment on the expression of HNRNPH1 in all three brain regions. These data suggest that both age and E2 contribute to changes in splicing-related factors, which could result in increased AS in the aged female brain.

Disclosures: C.L. Shults: None. **E. Pinceti:** None. **Y.S. Rao:** None. **T.R. Pak:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.12/E23

Topic: C.05. Aging

Support: NIH R01AG033605

Title: Mitogen Activated Protein kinase pathways are altered by loss of circulating estrogen in the aged female brain and heart in a region specific way

Authors: *E. PINCETI[1], C. L. SHULTS[2], Y. S. RAO[2], T. R. PAK[2];
[1]Cell. and Mol. Physiol., Loyola Univ. Chicago, Maywood, IL; [2]Cell and Mol. Physiol., Loyola UniversityChicago, Maywood, IL

Abstract: Aging and the coincident loss of circulating estrogens at menopause lead to increased risks for neurological and cardiovascular pathologies. Clinical studies show that estrogen therapy (ET) can be beneficial in mitigating these negative effects, in both the brain and heart, when it is initiated shortly after the perimenopausal transition. However, this same therapy is detrimental when initiated >10 years postmenopause. Importantly, the molecular mechanisms underlying this age-related switch in ET efficacy are unknown. Estrogen receptor β (ERβ) mediates both neuroprotective and cardioprotective functions of estrogens. Phosphorylation of steroid receptors of the same family as ERβ affects nearly every aspect of their signaling, including their ability to interact with coregulatory proteins and regulate gene transcription. This raises the possibility that aging and/or estrogens alter cellular signaling pathways resulting in altered ERβ-mediated physiological effects of ET. Indeed the cellular stress associated with aging has been shown to activate the Mitogen Activated Protein Kinases (MAPK) p38 and ERK. Our hypothesis is that age and estrogen deprivation following menopause alters the expression and activation of p38 and ERK kinases in the brain and heart. To test this hypothesis, we designed the following *in vivo* paradigm: surgically induced menopause was established in 18 mo. old rats through bilateral ovariectomy (OVX) and an acute dose of 17β-estradiol (E2; 2.5μg/Kg once/day x 3 days) or vehicle was administered at varying time points post-OVX (1 week, 4 weeks, 8 weeks, or 12 weeks). The mRNA expression and activation of p38 and ERK kinase in the hypothalamus, dorsal and ventral hippocampus and heart was measured using qRT-PCR and western blot analysis. The results showed that age and estrogen differentially regulate kinase activity in both the brain and heart, and the effects were also brain region specific. For example, the mRNA expression of the kinases tended to increase in all brain regions, yet their activity was differentially modulated. Overall, it is well understood that MAPKs signaling plays an integral role in aging, and their aberrant regulation might be involved in age-related disorders. Clinical studies show benefits of ET during early menopause but detrimental effects later, which might be reflective of changes in kinase expression and activation status. Supported by NIH R01AG033605 TRP

Disclosures: **E. Pinceti:** None. **C.L. Shults:** None. **Y.S. Rao:** None. **T.R. Pak:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.13/E24

Topic: C.05. Aging

Support: DC012125

NIDCD DC000151

Title: Age related changes in GABAergic inhibition in mouse auditory cortex, measured using *in vitro* flavoprotein autofluorescence imaging

Authors: *K. STEBBINGS[1], H. CHOI[1], A. RAVINDRA[1], J. TURNER[3], D. CASPARY[4], D. LLANO[2];
[2]Mol. and Integrative Physiol., [1]Univ. of Illinois At Urbana Champaign, Urbana, IL; [3]Psycology, Illinois Col., Jacksonville, IL; [4]Pharmacol., Southern Illinois Univ. Sch. of Med., Springfield, IL

Abstract: To examine aging-related changes in the earliest stages of auditory cortical processing, population auditory cortical responses to thalamic afferent stimulation were studied in young and aged mice. Cortical responses were measured using flavoprotein autofluorescence imaging, and aging-related changes in inhibition were assessed by measuring the sensitivity of the cortical responses to blockade of GABAA receptors using bath-applied SR95531. The maximum auditory cortical response to afferent stimulation was not different between young and old animals under control conditions, but older animals showed a lower sensitivity to GABA blockade with SR95531. Other parameters, such as EC50 and slope of the input-output curve differed between old and young animals at baseline, but showed equal sensitivities to SR95531. Hearing loss did not independently contribute to these aging-related changes, though cortical thickness was a strong predictor of most imaging variables. To determine if the observed differences between slices from young and aged animals were due to differences in slice health, the redox state of the slices was assessed by measuring the FAD+/NADH ratio using fluorescence imaging. We found that this ratio is highly sensitive to known redox stressors such as H2O2 and NaCN; however, no difference was found between old and young animals. These data extend previous work suggesting that auditory cortical inhibition diminishes with age and that the observed differences in the auditory cortex are not likely to tissue metabolic differences.

Disclosures: K. Stebbings: None. **H. Choi:** None. **A. Ravindra:** None. **J. Turner:** None. **D. Caspary:** None. **D. Llano:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.14/E25

Topic: C.05. Aging

Support: NINDS grant NS045260

Title: Ampakines stimulate dendritic growth in the hippocampus of middle-aged, environmentally enriched rats

Authors: *J. C. LAUTERBORN, L. C. PALMER, Y. JIA, B. HOU, D. T. PHAM, W. WANG, B. H. TRIEU, C. D. COX, S. KANTOROVICH, C. M. GALL, G. LYNCH;
Anat. & Neurobio., Univ. of California at Irvine, Irvine, CA

Abstract: Positive allosteric modulators of AMPA-type glutamate receptors (ampakines) up-regulate brain derived neurotrophic factor, and have been shown to rescue synaptic plasticity and reduce pathology in a neurodegenerative disorder rodent model. In the present study, we assessed 3 month (oral) treatments with the ampakine CX929 in middle-aged (MA) rats, housed in an enriched environment (EE), for effects on morphology and function of hippocampal field CA1 pyramidal cells and on measures of learning. The daily CX929 or vehicle treatments began at 9-10 months of age, and morphological effects were compared to vehicle-treated young rats. Dendritic branching and spine measures were assessed from 3D-reconstructions of Lucifer Yellow-filled cells. Sholl analyses of apical dendritic branching confirmed that aging results in substantial dendritic retraction, despite environmental enrichment. This effect was fully offset by CX929 treatment with values from ampakine (AK)-treated MA rats being comparable to those from young adults (young vs MA-vehicle: $p<0.0001$; young vs. MA-AK: $p>0.50$; MA-vehicle vs. MA-AK: $p<0.008$). Similar results were obtained for the basal dendrites and for measures of total dendritic length. While spine density did not differ between MA-vehicle and young rats, there was a significant increase in spine density in AK-treated rats relative to MA-vehicle and young groups. In a second set of MA rats, we examined the effects of 3 month CX929/EE treatment on synaptic physiology in CA1 stratum radiatum. Baseline synaptic physiology was subtly affected by CX929 treatment, in a manner consistent with greater dendritic branching. As anticipated from the morphological changes, the magnitude of LTP was substantially greater in MA-AK vs. MA-vehicle rats ($p=0.002$). Behaviorally, the ampakine group showed certain forms of long term memory that were lacking in MA-vehicle rats in tests conducted using novel, complex environments. Collectively, these findings provide evidence that long-term treatment with an ampakine can offset age-related losses in dendritic branches and that this is associated with improvements in plasticity and complex learning. Given that adverse side effects were not observed after prolonged treatment, the results are encouraging with regard to reducing age-related deterioration of brain.

Disclosures: **J.C. Lauterborn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder: The University of California (JL inventor). **L.C. Palmer:** None. **Y. Jia:** None. **B. Hou:** None. **D.T. Pham:** None. **W. Wang:** None. **B.H. Trieu:** None. **C.D. Cox:** None. **S. Kantorovich:** None. **C.M. Gall:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder: The University of California (CG inventor). **G. Lynch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder: The University of California (GL inventor).

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.15/E26

Topic: C.05. Aging

Support: Center for Nutrition, Learning, and Memory (Abbott Laboratories) project ZA69

Title: Long-term HMB supplementation ameliorates aging effects in the dendritic morphology of mPFC layer 5 pyramidal neurons in aged male and female rats

Authors: *D. G. KOUGIAS[1], S. O. NOLAN[2], T. KIM[2], W. A. KOSS[2], J. M. GULLEY[1,2], J. M. JURASKA[1,2];
[1]Neurosci. Program, Univ. of Illinois At Urbana-Champaign, Champaign, IL; [2]Psychology, Univ. of Illinois at Urbana-Champaign, Urbana-Champaign, IL

Abstract: Beta-hydroxy-beta-methylbutyrate (HMB), a supplement commonly used to maintain muscle in elderly and clinical populations, has been unexplored in the aging brain. In both healthy aging humans and rat models, cognitive deficits and dendritic shrinkage are associated with age-related changes in neurons within the prefrontal cortex (PFC). We have previously reported that aging male and female rats had a decrement in water maze performance, and HMB supplementation prevented that decrement in males alone. Our laboratory has also previously shown that aging male and female rats have smaller dendritic trees and fewer dendritic spines in the medial PFC when compared to young adults (Markham & Juraska, 2001). The current study explores the effects of relatively short- and long-term (7- and 31-week) oral HMB supplementation starting at 12 months of age in male and female rats on the dendritic tree of layer 5 pyramidal neurons in the medial PFC. At 11 months of age, female rats were ovariectomized to model human female aging following menopause, and male rats underwent sham surgeries. HMB was voluntarily ingested in a sucrose solution (450 mg/kg twice daily) while controls received a sucrose solution. Upon sacrifice, brains were obtained for Golgi Cox staining. The dendritic morphology of layer 5 pyramidal neurons was quantified and spines were counted. As expected, there was a retraction of dendritic material in both males and females in the apical and basilar trees as well as fewer spines in old age, compared to the middle-aged, controls. However, this dendritic loss did not occur in the HMB-treated rats of either sex in either dendritic branching or total number of dendritic spines. Thus, HMB ingestion for 31 weeks forestalled the effects of aging on the dendritic tree of this population of neurons.

Disclosures: D.G. Kougias: None. **S.O. Nolan:** None. **T. Kim:** None. **W.A. Koss:** None. **J.M. Gulley:** None. **J.M. Juraska:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.16/E27

Topic: C.05. Aging

Support: Chicago Biomedical Consortium postdoc award

NIH T32 AG20506

NIH AG008796

NIH AG017139

Title: Differential expression of calcium binding proteins (CBPs) in hippocampal subregions with aging

Authors: *D. SIMKIN, J. MA, A. HOFFMAN, M. OH, J. F. DISTERHOFT;
Physiology, Feinberg Sch. of Med., Northwestern Univ., Chicago, IL

Abstract: Extensive evidence suggests that dysregulation of Ca^{2+} homeostasis contributes to aging- and Alzheimer's-related impaired learning/memory associated with cognitive decline (Thibault et al., '07). For example, in aged CA1 hippocampal neurons, an enhanced activity dependent Ca^{2+} influx overwhelms endogenous Ca^{2+} buffers, resulting in higher free cytosolic Ca^{2+} concentrations and enhanced Ca^{2+}-dependent postburst-afterhyperpolarization as well as impaired synaptic plasticity and reduced intrinsic excitability (Tombaugh et al., '05; Disterhoft and Oh, '06). Despite reports of cell-permeable Ca^{2+}-chelators (which reduce the impact of increased Ca^{2+} influx) ameliorating aging-related spatial learning deficits and increasing cellular excitability in rats (Tonkikh et al., '06), associated systemic side effects prevent their therapeutic use. However, cells possess effective homeostatic mechanisms to regulate Ca^{2+}-signaling and intracellular concentration, such as Ca^{2+}-buffering (through chelation) by Ca^{2+}-binding proteins (CBPs). Recently, our laboratory determined that increased Ca^{2+} influx is partially countered by enhanced endogenous intracellular Ca^{2+}-buffering capacity in aged CA1 hippocampal pyramidal neurons (Oh et al., '13). As not all aged subjects experience impairments in memory, cognitive decline may not represent a "more aged" phenotype, but rather be associated with specific neuroproteomic changes compounding age-related alterations. Thus the increased Ca^{2+} buffer capacity may represent an adaptive cellular mechanism to compensate for the increased Ca^{2+} influx observed in aged animals. Understanding how age-related changes in the hippocampal neuroproteome contribute to altered neuronal excitability with either impairment or the preservation of memory function with age are critical objectives that remain to be achieved. Thus we examined changes of known Ca^{2+}-associated buffering proteins that can impact intrinsic neuronal excitability using MicroWestern array (MWA) technology in young (2-4 mo) and aged (29-31 mo) rats. This innovative technology allows for the expression of hundreds of proteins to be semi-quantitatively profiled at one time, therefore identifying a wide range of target proteins

that could potentially contribute to the preservation of memory function with age. Several classical CBPs were found to be reduced with aging in CA1 hippocampal tissue such as calbindin, hippocalcin and calreticulin. We are investigating the age-related differential expression of those and other CBPs in the hippocampal subregions (CA1, CA3 and dentate gyrus).

Disclosures: D. Simkin: None. **J. Ma:** None. **A. Hoffman:** None. **M. Oh:** None. **J.F. Disterhoft:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.17/E28

Topic: C.05. Aging

Support: 2R01AG033649-06

Title: Acute insulin on Ca^{2+} homeostasis and glucose utilization in single hippocampal neurons

Authors: *S. MAIMAITI[1], K. L. ANDERSON[1], J. POPOVIC[1], L. BREWER[1], Z. MAJEED[2], H. FRAZIER[1], N. M. PORTER[1], P. W. LANDFIELD[1], O. THIBAULT[1];
[1]Pharmacol. and Nutritional Sci., [2]Biol., Univ. of Kentucky, LEXINGTON, KY

Abstract: Recent work from our lab identified that intranasal insulin improves memory in the aged F344 rats (Maimaiti et al., 2015). It is well documented that neurons in the brain are insulin sensitive. It has also been shown that brain insulin sensitivity may be reduced in Alzheimer's disease (AD) and/ or aging. Further, clinical trials have repeatedly shown that intranasal insulin can significantly improve memory not only in AD patients, but also in younger healthy individuals. However, the mechanism whereby insulin can alter neuronal function is not clear. While prior work has highlighted changes in AMPA and NMDA receptors, very little work has focused on voltage-gated calcium channels/ currents (VGCCs) as a target of insulin action in the brain. Earlier studies from several labs have looked at intracellular Ca2+ as a key neuronal molecular regulator of hippocampal-dependent memory. Elevated intracellular Ca2+ levels in hippocampal neurons have been shown in aged animals with poor spatial memory. Recently, we have shown that insulin can reduce the Ca2+-dependent afterhyperpolarization (AHP) in hippocampal neurons in both young and aged animals (Maimaiti et al., 2015). The goal of the present work was to test the hypothesis that insulin reduces VGCCs thereby reducing the AHP. We used whole cell patch clamping, Ca2+ imaging (Bis-Fura-2) and glucose imaging techniques (2-NBDG) to measured Ca2+ currents, intracellular Ca2+ concentration and glucose utilization in 13-17 DIV mixed hippocampal neurons in culture. Active, boiled (10 nM) glulisine insulin Apidra® (rapid-acting, zinc-free form of insulin) and human recombined insulin were tested for

acute effects on neuronal VGCCs, Ca2+ levels and glucose utilization. Results show that neurons treated with 10 nM Apidra® had significantly reduced Ca2+ currents. This indicates the mechanism of insulin-mediated memory improvement could be due to reduced calcium flow through VGCCs and the ensuing reduction in the AHP (Pancani et al., 2013).

Disclosures: S. Maimaiti: None. **K.L. Anderson:** None. **J. Popovic:** None. **L. Brewer:** None. **Z. Majeed:** None. **H. Frazier:** None. **N.M. Porter:** None. **P.W. Landfield:** None. **O. Thibault:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.18/E29

Topic: C.05. Aging

Support: 2R01AG033649-06

Title: Acute intranasal insulin in young and aged F344 rats: signaling and MRI brain changes

Authors: *K. L. ANDERSON[1], S. MAIMAITI[1], Z. R. MAJEED[2], H. FRAZIER[1], V. BAKSHI[1], L. D. BREWER[1], N. M. PORTER[1], A.-L. LIN[1], O. THIBAULT[1];
[1]Pharmacol. and Nutritional Sci., [2]Biol., Univ. of Kentucky, Lexington, KY

Abstract: In an attempt to combat decreased insulin signaling in the brain of Alzheimer's disease (AD) patients, several groups have used intranasal insulin in clinical and pre-clinical settings. Results show improved memory in young volunteers, patients with mild cognitive impairment, as well as in animal models of aging, diabetes or AD. While some studies have linked insulin signaling in the brain with tau or Abeta clearance mechanisms, with changes in excitatory neurotransmitters or synaptic plasticity, reductions in Ca2+, or improved hypothalamic perfusion, it is not clear how intranasal insulin alters functional communication, improves learning, or facilitates memory retrieval, particularly in animal model of aging. Here we characterized the impact of acute (single dose) intranasal insulin across different brain regions at different times following delivery. We tested the impact of Apidra® on 8 different brain regions in young (3-4 months) and aged (21-24 months) F344 rats (n=12/age group). Animals received a unilateral dose of Apidra® (0.0715 IU/rat) or saline. Olfactory bulbs and brains were removed at 30, 60, or 120 min after delivery. Insulin activity was quantified with a focus on the canonical Akt and pAkt protein expression pathway with Western blots. The pAkt:Akt ratio significantly increased at 30m in the right olfactory bulb, which was exposed to insulin, and went down to normal levels by 120 min. Dorsal and ventral signaling increases were noted at 60 and 120 min post delivery. Acute Apidra® was also tested on memory retrieval on the Morris water maze in young and aged F344 (n=20/age group) following a single dose. Saline

delivery lasted for 8 days with MWM training starting on the fifth day. On the 9th day, half the animals received Apidra. In a subset of these animals, neurotransmitter levels were determined by MR spectroscopy (MRS) and cerebral blood flow was measured by MRI. Our work highlights new details on acute intranasal insulin delivery, and in particular its' impact on memory retrieval compared to acquisition (Maimaiti et al., 2015). Changes in MRS and MRI signaling also helped rule out effects mediated solely by blood flow dynamics. These results also provide insights on improved timing between insulin delivery and behavioral characterization.

Disclosures: K.L. Anderson: None. **S. Maimaiti:** None. **Z.R. Majeed:** None. **H. Frazier:** None. **V. Bakshi:** None. **L.D. Brewer:** None. **N.M. Porter:** None. **A. Lin:** None. **O. Thibault:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.19/E30

Topic: C.05. Aging

Support: NIA Grant 2R01 AG033649-06

Title: Characterization of a truncated human insulin receptor signaling

Authors: *Z. R. MAJEED[1], H. N. FRAZIER[2], K. HAMPTON[2], S. MAIMAITI[2], K. L. ANDERSON[2], J. POPOVIC[2], L. B. BREWER[2], S. D. KRANER[2], C. M. NORRIS[2], N. PORTER[2], R. J. CRAVEN[2], O. THIBAULT[2];
[1]Biol., Univ. of Kentucky, Dept. of Biol., Lexington, KY; [2]Pharmacol. and Nutritional Sci., Univ. of Kentucky, Lexington, KY

Abstract: Insulin signaling is indispensable for key metabolic pathways in the periphery, and recently several studies have demonstrated that insulin signaling is also important for brain function. Early stage clinical trials report on the positive impact of intranasal insulin on memory recall in young subjects and in patients with mild cognitive decline or Alzheimer's disease (AD). However, the underlying molecular mechanisms for the actions of insulin signaling on brain cognitive function are not well understood. Here we sought to investigate the role of insulin in neuronal physiology by overexpressing a constitutively active human insulin receptor (Lebwohl et al., 1991) in rat pheochromocytoma (PC12) and HEK 293 embryonic kidney cells to obtain insights into the trafficking of the protein, as well as its' activity and sensitivity to exogenous insulin. Cells were transfected by electroporation with pCI-ires-dsRed (a mammalian expression plasmid containing a gene encoding a red fluorescence protein) or pCI-IRβ-ires-dsRed (the construct with a truncated human insulin receptor beta subunit (IRβ) transcript). The vector was originally test in HEK293 cells. The expression of human IRβ receptor in PC12 cells was

corroborated by the expression of red fluorescent protein (dsRed) since IRβ and dsREd were co-expressed under the control of the same promotor (CMV). The expression level and effect of IRβ receptor overexpression on insulin signaling is confirmed by performing immunoblots, using antibody against tagged IRβ, and measuring pAkt/Akt ratio, respectively, because Akt is one of the main downstream signaling molecule targets for the insulin receptor signaling pathway. Our data show that overexpression of insulin receptor enhances neurite outgrowth in PC12 cells and increases the pAkt/Akt ratio. Biotinylation experiments also revealed insertion of the protein in the plasma membrane. Further studies were performed to analyze the sensitivity of the truncated insulin receptor activation to acute insulin application. This initial characterization of the protein provides insights into future intervention approaches to combat reduced insulin signaling in AD and/ or aging.

Disclosures: Z.R. Majeed: None. **H.N. Frazier:** None. **K. Hampton:** None. **S. Maimaiti:** None. **K.L. Anderson:** None. **J. Popovic:** None. **L.B. Brewer:** None. **S.D. Kraner:** None. **C.M. Norris:** None. **N. Porter:** None. **R.J. Craven:** None. **O. Thibault:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.20/E31

Topic: C.05. Aging

Support: Fundação para a Ciência e Tecnologia, Portugal

SFRH/BD/52228/2013 (MTF)

ATIP/AVENIR program (Centre national de la recherche scientifique - CNRS)

Fondation pour la Recherche Médicale (PP)

Investigator FCT (LVL)

Title: Neuronal adenosine A2A receptor overexpression affects AMPA and NMDA currents in CA1 hippocampal neurons

Authors: *L. V. LOPES[1], M. TEMIDO-FERREIRA[1], J. E. COELHO[1], D. G. FERREIRA[1,2], T. F. OUTEIRO[2], M. BADER[3], H. MARIE[4], P. A. POUSINHA[4];
[1]Inst. de Medicina Molecular, Fac Med. Lisbon, Lisbon, Portugal; [2]Dept. of Neurodegeneration and Restorative Res., University Medizin Göttingen, Germany; [3]Max-Delbrück-Center for Mol. Med. (MDC), Berlin, Germany; [4]Inst. de Pharmacologie Moléculaire et Cellulaire (IPMC), Ctr. Natl. de la Recherche Scientifique (CNRS), Université de Nice Sophia Antipolis, France

Abstract: Aging and Alzheimer's disease (AD) are associated with cognitive impairments, accompanied by structural and functional alterations in the hippocampus. There is compelling evidence of hippocampal upsurge of adenosine A2A receptors (A2AR) associated to cognitive deficits. Blockade of A2AR in experimental models mimicking aging or AD prevent, or even revert, hippocampus-related impairments (Batalha et al, 2013; Laurent et al, 2015, Mol. Psychiatry). This suggests that dysregulation of hippocampal A2AR function may drive part of the detrimental processes leading to aging and AD. However, the underlying mechanisms are still unknown. We generated transgenic rats overexpressing the human A2AR under the control of the CAMKII promoter [tg (CAMKII-hA2AR)], inducing a specific neuronal overexpression. In order to characterize the impact on neuronal excitability and synaptic transmission, we performed whole-cell patch-clamp recordings in CA1 hippocampal neurons from transgenic versus wildtype (WT) animals. We measured Paired Pulse Ratios (PPRs), in the range of 50 - 200ms intervals: a PPR facilitation was observed in neurons from WT animals at all intervals, more evident for the shorter intervals (n=10). The magnitude of PPR was reduced in tg(CAMKII-hA2AR) rats when compared to the WT neurons (n=10; $P<0.05$), albeit maintaining the same facilitatory profile. These effects were completely rescued by SCH 58261 (50 nM). We assessed the changes in AMPAR and NMDAR contribution, by quantifying the AMPAR/NMDAR which we found to be decreased in tg(CAMKII-hA2AR) animals (n=19-21; $P<0.05$). Current-voltage (I-V) relationships in pharmacologically isolated NMDAR or AMPAR EPSCs revealed that AMPAR activation was decreased (n=7; $P<0.05$), while the I-V profile of NMDAR was increased (n=7; $P<0.05$). Such pre- and post-synaptic effects are not due to neuronal excitability, since no differences were observed between WT and tg(CAMKII-hA2AR) animals in the excitability parameters. Altogether these data show that neuronal A2AR overexpression, per se, shares some electrophysiological features observed in both AD models and aged animals, strongly suggesting that A2AR overexpression might be one of the key events in the AD- and aging-associated glutamatergic synaptic dysfunction.

Disclosures: **L.V. Lopes:** None. **M. Temido-Ferreira:** None. **J.E. Coelho:** None. **D.G. Ferreira:** None. **T.F. Outeiro:** None. **M. Bader:** None. **H. Marie:** None. **P.A. Pousinha:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.21/E32

Topic: C.05. Aging

Support: NIH Grant P01-AG000001

NIH Grant R01-AG043640

NIH Grant R01-AG042512

Graduate Program for Neuroscience

Title: Phagocytic macrophages are associated with processes involved in age and cognitive decline

Authors: *E. SHOBIN[1,2], T. L. MOORE[1], D. L. ROSENE[1];
[1]Anat. and Neurobio., [2]Grad. Program for Neurosci., Boston Univ., Boston, MA

Abstract: The rhesus monkey is a good model of normal aging as it is free from Alzheimer's disease yet shows age-related decline in cognitive domains similar to humans (Moss, et al. 2007). Previous studies in the monkey have shown that neurons are not lost but instead, myelin pathology occurs and correlates with cognitive impairments (Bowley, et al. 2010). In exploring possible mechanisms our prior studies have shown that microglia become chronically pro-inflammatory (M1) with age and this activation is associated with cognitive decline (Sloane, et al. 1999). To determine if activated microglia in the monkey become phagocytic in response to myelin degradation we used antibody to Galectin-3 (Gal-3), a lectin selectively up-regulated in phagocytic macrophages and essential for normal phagocytosis as Gal-3 knockout mice have impaired phagocytosis (Sano, et al. 2003). We used brain sections from young (6-10 years, n = 7) and old (20-30 years, n = 14) behaviorally tested rhesus monkeys and quantified Gal-3 immunostaining in frontal white matter regions. There was a significant increase in Gal-3+ cells in old compared to young monkeys ($p < 0.001$). Additional analysis in only the old monkeys showed that Gal-3+ cell density was significantly greater in those cognitively impaired compared to those relatively cognitively spared ($p < 0.05$). To further assess the relationship of phagocytosis to myelin damage, sections are being processed with double label IHC for damaged myelin basic protein (dMBP) and Gal-3. Other sections are being processed with double label IHC to determine the proportion of microglia positive for the LN3 marker for M1 microglia that are also Gal-3+ phagocytic and how this relates to the magnitude of myelin damage.

Disclosures: **E. Shobin:** None. **T.L. Moore:** None. **D.L. Rosene:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.22/E33

Topic: C.05. Aging

Support: CONACYT Grant 176393

CONACYT Grant 179506

Title: Prolactin receptor signaling mitigates retinal function deficiency associated with ageing

Authors: *E. ARNOLD, S. THEBAULT, G. MARTÍNEZ DE LA ESCALERA, C. CLAPP; Neurobio. Inst., Natl. Autonomous Univ. of Mexico, Queretaro, Mexico

Abstract: Age retinal degeneration is characterized by the progressive destruction of retinal cells, causing deterioration and eventual loss of vision. We explored whether the hormone prolactin (PRL) provides trophic support to aged retinal cells, thus protecting the retina from age related degeneration. Retinal function, apoptosis, gliosis, and neurotrophin expression were assessed by electroretinogram, TUNEL, GFAP immunohistochemistry, and real-time PCR, respectively, in for PRL receptor null mice. Lack of PRL signaling associates with photo responsive dysfunction and gliosis that correlated with the down-regulation of neurotrophins (bFGF, GNDF, and BDNF) and antioxidant glutathione S-transferase μ-type 2 expression. Most of these effects were exacerbated in aged (9 month-old) compared with young (3 month-old) mice. This study unveils PRL as a trophic factor regulating neuronal-glia cell interactions in the aged retina, and its potential therapeutic value against age related retinal degeneration.

Disclosures: E. Arnold: None. **S. Thebault:** None. **G. Martínez de la Escalera:** None. **C. Clapp:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.23/E34

Topic: C.05. Aging

Support: BBSRC grant (BB/G015988/1) to Saffrey MJ and Ranson RN

Title: Age-related changes in substance P immunoreactivity and motoneuron morphological alterations in lumbosacral spinal nuclei

Authors: H. W. S. TSANG[1], M. ZHANG[1], G. BLACK[1], M. J. SAFFREY[2], *R. N. RANSON[1]; [1]Applied Sci. (Biomedical Sciences), Northumbria Univ., Newcastle Upon Tyne, United Kingdom; [2]Dept. of Life, Hlth. and Chem. Sci., Open Univ., Milton Keynes, United Kingdom

Abstract: Incontinence and sexual dysfunction increase in the elderly. In humans, voluntary control is partially mediated by Onuf's nucleus located in lumbosacral spinal cord (LS-SC). Its homolog in rodents is divided into two spinal nuclei, the dorsolateral nucleus (DLN) and the spinal nucleus of the bulbocavernosus muscle (SNB). In these nuclei are motoneurons (MNs) that directly innervate the ischiocavernosus and external urethral sphincter (DLN), and those that innervate the levator ani, bulbocavernosus muscle, and external anal sphincter (SNB). Synaptic contacts onto SNB-MNs are reduced in aged (24 months) rats. Furthermore, a reduction in the number of MNs in 22 month-old rats has been reported. Here, we present immunofluorescence data on age-related changes in the amount of substance P (SP) in the LS-SC of C57BL/6 male

mice. In addition, we present data on SP inputs to MNs of the DLN/SNB, and semi-quantitative ultrastructural data on age-related morphology of DLN-MNs. SP immunoreactivity in the DLN, SNB, and dorsal horn where lower urinary tract sensory input is received, decreased in aged (24-25.5 month old) mice. However, SP immunoreactivity increased significantly in all three regions from aged to very aged (30-31 month-old) animals. Somatic SP appositions to DLN-MNs also increased significantly in very aged mice. Immunolocalization of SP terminals to DLN-MNs showed symmetric synaptic contacts. The cross-sectional area of DLN-MNs increased by 22% from 3-4.5 months to 24-25.5 months. A notable alteration to the ultrastructural morphology of DLN-MNs from 4 - 32 month-old animals was a progressive increase in electron dense organelles, which were likely lipofuscin-containing structures. These lipofuscin bodies were rare in 4 month-old material, but were larger, clustered and more frequently seen in 32 month-old. Rough endoplasmic reticulum (RER), polyribosomes and mitochondria were the predominant organelles/structures in the perinuclear region in 4 month-old DLN-MNs. By 32 months, lipofuscin dominated the perinuclear region. In MNs of the very aged, membranes of the Golgi apparatus, cristae and outer membrane of mitochondria were generally slack in appearance. In addition, the nuclear membrane displayed frequent deep invaginations that partially enveloped cytoplasmic contents including RER and polyribosomes. Together, age-related changes seen in this study may contribute to dysfunctional alterations in the ability to maintain continence and facilitate sexual reflexes.

Disclosures: **H.W.S. Tsang:** None. **M. Zhang:** None. **G. Black:** None. **M.J. Saffrey:** None. **R.N. Ranson:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.24/E35

Topic: C.05. Aging

Support: University of Exeter

Title: Aging increases the activity of neurons in the bed nucleus of the striata terminalis (BNST)

Authors: *H. E. SMITHERS[1], J. BROWN[1], J. TERRY[1], A. RANDALL[1,2];
[1]Univ. of Exeter, Exeter, United Kingdom; [2]Univ. of Bristol, Bristol, United Kingdom

Abstract: The BNST is a nucleus in the limbic forebrain that plays a role stress, fear and anxiety. The BNST is also sexually dimorphic and has been implicated in determination of sexual identity. As well as acting a major output pathway for the amydala, this nucleus is thought to act as a relay for cortical and limbic control of hypothalamic function. Aging is reported to alter neural activity in various brain regions including the prefrontal cortex and hippocampus,

however, to our knowledge, the effects of healthy aging on electrophysiological properties of BNST neurons has not been examined previously. This study examined the effect of age the intrinsic properties of BNST neurons in cohorts of female mice. *In vitro* patch clamp recordings were made from 300µM coronal brain slices prepared from either adult female mice of 3-5 months (young) or 29 months (Old). The resting membrane potential did not differ between the cohorts (young -69 ± 2 mV, Old -67 ±2 mV) however a far higher proportion of the cells recorded from aged animals (62%, n=50) fired at rest compared to the younger cells (42%, n=56, Chi Squared p = 0.001). In firing cells maintained spontaneous firing rates as high as 10 Hz were observed, although the median value was circa. 4 Hz in both cohorts. At a set membrane pre-stimulus potential of -80 mV no differences in a number of passive membrane properties or action potential properties were observed. These include input resistance (Young 445 ± 38 MΩ, Old 501 ± 49 MΩ), membrane time constant (Young 29 ± 2 ms, Old 29 ± 2 ms) and AP Zenith (17 ± 1 mV, Old 17 ± 2 mV). To examine excitability, incremental depolarizing current stimuli (5-80 pA) lasting 500 ms were applied to BNST neurons at -80 mV. As the amplitude of the stimulus was increased both the probability and the rate of AP production rose. Firing Rates in the aged cohort were significantly higher than in cells from younger animals (2-way ANOVA p = 0.011). A higher proportion of cells from the older animals fired in response to each depolarising step. This was most evident with the +20 pA step (Young 15/55 cells, Old 22/16 firing). In combination with the increased spontaneous firing at rest, these data indicate aging heightens excitability of BNST neurones and likely impacts their functions such as modulation of the HPA axis.

Disclosures: H.E. Smithers: None. **J. Brown:** None. **J. Terry:** None. **A. Randall:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.25/E36

Topic: C.05. Aging

Title: Bexarotene treatment rescues aging-related loss of synaptic proteins in a neuronal LRP1-dependent manner

Authors: *M. TACHIBANA, M. SHINOHARA, Y. YAMAZAKI, C.-C. LIU, J. ROGERS, G. BU, T. KANEKIYO;
Mayo Clin., Jacksonville, FL

Abstract: Apolipoprotein E (apoE) plays a critical role in maintaining synaptic integrity by transporting cholesterol to neurons through its cell surface receptor, the low-density lipoprotein receptor related protein-1 (LRP1). Although controversial, Bexarotene (Bex), a retinoid X receptor (RXR) agonist, has been reported to have potential beneficial effects on cognition by

increasing brain apoE levels and lipidation. However, it remains unclear if Bex treatment can restore synaptic function in aged mice and whether neuronal LRP1 is involved in the pathway. To investigate the potential effects of Bex, forebrain neuron-specific LRP1 knockout (nLRP1-KO) and littermate control mice were administered Bex-containing diet (100mg/kg/day) or control diet at the age of 20-24 months for 8 weeks. We found that upon Bex treatment, levels of brain apoE and ABCA1 were significantly increased both in control and nLRP1-KO mice. While levels of synaptic proteins, synaptophysin and PSD95, were decreased during aging, they were restored in the cortex and hippocampus of Bex-treated mice compared to control mice. Western blotting also showed that PSD95, GluR1 and NMDAR1, which are key postsynaptic proteins that regulate synaptic plasticity, were increased by Bex treatment in the brains of control mice. Interestingly, Bex treatment did not affect the levels of these proteins in nLRP1-KO mice. These results indicate that the beneficial effects of Bex on synaptic integrity depend on the presence of neuronal LRP1. However, Bex treatment resulted in weight loss by 10-15% as well as prominent hepatomegaly in both control and nLRP1-KO mice, which is likely due to hepatic dysfunction. Furthermore, immunohistochemical analysis indicated that GFAP-positive astrocytes and Iba-1 positive microglia were significantly increased in Bex-treated mice compared to control mice, indicating exacerbated neuroinflammation. Taken together, our results demonstrate that apoE targeted treatment by RXR agonist, has a potential effect on synapses through LRP1 during aging; however, the potential therapeutic value of Bex might be limited due to harmful side effects.

Disclosures: **M. Tachibana:** None. **M. Shinohara:** None. **Y. Yamazaki:** None. **C. Liu:** None. **J. Rogers:** None. **G. Bu:** None. **T. Kanekiyo:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.26/E37

Topic: C.05. Aging

Support: NIH Grant NS070825

NIH Grant AG024827

NIH Grant AG034464

NIH Grant AG039477

Funding from the Agining Institute of University of Pittsburgh/UPMC

Title: An enriched environment modulates factors associated with healthy brain aging in rats

Authors: *M. J. ZIGMOND[1], F. AMBROSIO[2], S. L. CASTRO[1], J. D. JAUMOTTE[1], D. L. KOROL[3], L. A. NEWMAN[3], L. H. SANDERS[1], R. J. SMEYNE[4], A. D. VALLEJO[1]; [1]Neurol., [2]Physical Med. & Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; [3]Biol., Syracuse Univ., Syracuse, NY; [4]Dept. of Developmental Neurobio., St. Jude children's Res. Hosp., Memphis, TN

Abstract: The sedentary and environmentally impoverished lifestyle that characterizes many older individuals within modern society may play a critical role in the motor, cognitive, and emotional decline that often occurs during advanced years. Clinical and animal studies suggest that an enriched environment (EE) can increase socialization, motor learning, and mobility, and promote changes in the brain that appear to facilitate healthier aging, including neurogenesis, reduced cellular stress, synaptogenesis, and increased neurotrophic factor levels. We are currently assessing the behavioral, physiological, and neurobiological impact of EE in older rats using a behavioral test battery and biochemical, molecular biological, and anatomical methods. F344/BN male rats 18 month old at the outset of our studies were housed individually for at least 4 mo in a standard shoebox cage (SE) or in groups of 6 in an EE (1 m x 1 m x 1 m) containing running wheels, tunnels, platforms, and toys. All rats were exposed to a 12:12 light-dark cycle with ad libitum food and water, and videotaped for 14 hours each day, including during the dark cycle. Although physical activity was low relative to that of younger rats housed under similar conditions, we observed a good deal of exploration, climbing, playing, and social interactions. Body weight increased significantly in the EE rats, but not in the SE rats. After 4 months, rats were euthanized, the brain, peripheral tissues, and blood collected, and the brain dissected into several regions. Several biochemical, histological, and body composition assessments are currently being made. For example, post-mortem analysis of muscle showed that citrate synthase activity and fat content in the gastrocnemius were similar in rats from both conditions, supporting the evidence from video records that rats in EE conditions were not aerobically active. In addition, compared to SE rats, EE rats showed a 3.8-fold increase in BDNF in the hippocampus and a 60% increase in the ratio of dopamine (DA) metabolites to DA in the striatum, consistent with an increase in striatal DA turnover. Additional assays are ongoing, including those for neurotrophic factors, muscle composition, mitochondrial damage, inflammation, and gene expression. Thus far our results suggest that housing in an EE produces significant changes consistent with increased neuroplasticity even in the absence of increased aerobic activity, emphasizing the likely importance of other behavioral components of environmental enrichment. We hypothesize that these changes are associated with an increase in brain health and improved behavioral capacity during aging.

Disclosures: M.J. Zigmond: None. **F. Ambrosio:** None. **S.L. Castro:** None. **J.D. Jaumotte:** None. **D.L. Korol:** None. **L.A. Newman:** None. **L.H. Sanders:** None. **R.J. Smeyne:** None. **A.D. Vallejo:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.27/E38

Topic: C.05. Aging

Support: JSPS KAKENHI Grant number 24590116

Title: Enriched environment improves micturition activity in awake freely moving aged mice

Authors: *F. SOEDA[1], N. GOTO[1], S. SAMESHIMA[1], S. MISUMI[1], K. TAKAHAMA[2,3]; [1]Dept. of Envrn. and Mol. Hlth. Sci., Grad. Sch. of Pharmaceut. Sci., [2]Res. Inst. for Drug Discovery., Sch. of Pharm., Kumamoto Univ., Kumamoto, Japan; [3]Fac. of Hlth. Sci., Kumamoto Hlth. Sci. Univ., Kumamoto, Japan

Abstract: We previously reported that enriched environment (EE) promotes micturition activity of freely moving normal mice. However, it is not known about the effect of EE on disorder of micturition function associated with aging in mice. In this study, we examined whether or not rearing in EE affects micturition activity in senescence-accelerated mouse prone 8 (SAMP8) mice. Male SAMP8 (32 weeks old) mice were reared in standard environment (SE) or EE for 5 weeks. As a control, senescence-accelerated resistant (SAMR1) mice were reared in SE. Micturition activity of freely moving mice was measured by using an apparatus developed by us. Mice were fed ad libitum and were kept under a 12:12-h light-dark cycle. In the light period, SAMP8 reared in the SE showed significant increase in total voided volume, single voided volume, mean flow rate and maximum flow rate of urine when compared with SAMR1. On the other hand, the mice reared in EE showed significant decrease in single voided volume, mean flow rate and maximum flow rate of urine when compared with that reared in SE. In the dark period, there was no significant difference in these parameters among the three groups. Volume of water intake during the measurement of micturition activity did not change among the three groups. Preliminary microarray analysis of the micturition centers of SAMP8 found that the level of some molecules may be changed when reared in EE compared to SE. The results suggest that rearing in EE may improve micturition activity in the resting period of SAMP8 mice. Further studies are needed to identify the molecules associated with amelioration of micturition activity in SAMP8 reared in EE.

Disclosures: F. Soeda: None. **N. Goto:** None. **S. Sameshima:** None. **S. Misumi:** None. **K. Takahama:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.28/E39

Topic: C.05. Aging

Support: NIH Grant AG 039818

Title: Daily exposure to environmental novelty in young mice promotes corticostriatal and behavioral flexibility in old age

Authors: *S. L. HONG[1], S. J. BARTON[2], G. V. REBEC[2];
[1]Biomed. Sci., Ohio Univ., Athens, OH; [2]Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: We assessed early rearing conditions on aging-related changes on neuronal signaling patterns in motor cortex and dorsal striatum as mice explored a plus-shaped maze. Mice were raised in one of the following four conditions beginning at ~4 weeks of age: 1) Isolated Empty Cage (IHEC); 2) Isolated Running Wheel (IHRW); 3) Enriched Environments - Static (EEST); and 4) Enriched Environment - Dynamic (EEDY). Both enriched groups included toys and other mice. For EEDY, the toys and the location of sources of food and water were changed daily, but remained constant for EEST. All mice remained in their respective environments for 25 weeks followed by single housing in empty cages for the remainder of the experiment. Beginning at ~40 weeks of age, all mice were tested at regular monthly intervals in a plus-shaped maze in which we measured the number and pattern of arm choices and recorded local field potentials (LFPs). Unpredictability in cortical and striatal LFPs was assessed by approximate entropy (ApEn). Corticostriatal synchrony was assessed by obtaining phase locking values (PLV) at four time points relative to the center choice point of the plus maze: A) 2s-1s prior; B) 1s prior-choice point; C) choice point-1s after; and D) 1s-2s after. Our results indicate a unique plasticity in EEDY cortical and striatal ApEn levels, which were elevated relative to IHRW and IHEC. In old age, higher PLV values indicative of greater corticostriatal synchrony were observed in comparison to the IHEC group. Behaviorally, the IHEC and EEST groups demonstrated a reduced ability to adapt to repeated exposures to the plus maze; arm-choice patterns and number of arm entries were unchanged over time. Collectively, our results highlight the benefits of early exposure to daily environmental novelty in the maintenance of healthy brain function and behavioral flexibility in aging; this effect persists even months after environmental changes are withdrawn.

Disclosures: **S.L. Hong:** None. **S.J. Barton:** None. **G.V. Rebec:** None.

Poster

305. Aging: Animal and Cellular Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 305.29/E40

Topic: C.05. Aging

Support: NIH Grant AG040261

Title: Treadmill exercise attenuates aging-related bradykinesia in aged rats: potential involvement of increased nigral glial cell line-derived neurotrophic factor family receptor-alpha 1 (GFR-α1) expression and dopamine tissue content

Authors: *J. C. ARNOLD[1], M. F. SALVATORE[1,2];
[1]Pharmacology, Toxicology, & Neurosci., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA;
[2]Pharmacol. & Neurosci., Univ. of North Texas Hlth. Sci. Ctr. at Ft. Worth, Ft. Worth, TX

Abstract: Given the burgeoning increase in our elderly population, lifestyle strategies that mitigate aging-related impairments are essential. Bradykinesia, a cardinal symptom of Parkinson's disease (PD), also affects up to 30% of the elderly population. Exercise may improve locomotor deficits in PD models and patients, but the neuroanatomical and molecular basis for these effects have not been pinpointed to striatal dopamine (DA) recovery. In PD, striatal DA loss exceeds 80% at onset of bradykinesia, but in aging, striatal DA loss has not ever been reported to exceed 50%. However, in aging and PD alike, the onset of bradykinesia may be associated with 50% DA loss in the substantia nigra (SN). Here, we hypothesized that an established treadmill exercise regimen could attenuate aging-related bradykinesia (ARB) in conjunction with increased DA and the glial cell line-derived neurotrophic factor (GDNF) receptor, GDNF family receptor-alpha 1 (GFR-α1) in the SN. The rationale for this hypothesis is based on observations that striatal infusion of GDNF in aging models increases locomotor activity and DA in the SN, but not striatum. Exercise may also increase GDNF expression, and exogenous GDNF may increase GFR-α1 expression in the SN. We have also reported that GFR-α1 decreases only in the SN in aging, and replenishing the quantity of GFR-α1 lost due to aging increases locomotor activity in combination with increased DA and tyrosine hydroxylase (TH) expression in SN, but not striatum, in aged rats. Using our treadmill exercise regimen, we assessed the impact of short- and long-term exercise on ARB and GDNF signaling in aged rats. Our results demonstrate that two rounds of our exercise regimen increased GFR-α1 expression and DA tissue content in SN of aged rats: a result that reflects the previously reported effect of exogenous GDNF. Notably, a repeated regimen of long-term exercise followed by an equal amount of rest eventually attenuated ARB when compared to non-exercise rats. These studies may be applicable in PD models, in that reduction of age-related loss of DA in the SN may be an important mechanism of reducing bradykinesia. Finally, our work may suggest that a therapeutic strategy that reduces ARB and increases DA synthesis in the SN may be a sufficient means to target bradykinesia, particularly in those who may be physically unable or unwilling to exercise.

Disclosures: J.C. Arnold: None. **M.F. Salvatore:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.01/E41

Topic: C.06. Developmental Disorders

Support: Banting Postdoctoral Fellowship

Autism Research Training Grant

Title: On the spectrum: sensory perception influences socio-communication in ASD

Authors: *R. A. STEVENSON[1], M. SEGERS[3], M. D. BARENSE[2], S. FERBER[2];
[2]Dept. of Psychology, [1]Univ. of Toronto, Toronto, ON, Canada; [3]Dept. of Psychology, York Univ., Toronto, ON, Canada

Abstract: Autism Spectrum Disorder (ASD) has become increasingly common, now at one in 68 births. One of the primary factors in ASD is difficulty in social communication. While socio-communication issues have long been included in the diagnostic criteria, emerging theories posit that these difficulties may arise from divergent developmental cascades that stem from atypical sensory perception during development. In short, most higher-order cognitive functioning, including social communication, are dependent upon sensory perception. Differences in sensory perception, particularly throughout development, may thus lead to differences in social communication. We explored this hypothesis through a series of experiments in individuals with and without ASD focusing on the ability to integrate individual pieces of sensory information into coherent, unified representations - a process known as perceptual binding. Using four well characterized perceptual paradigms, we measured individuals' abilities to perceptually bind social and non-social sensory information within the visual system (using the composite-face effect and the composite-letter effect, respectively), as well as across the visual and auditory system (using the McGurk effect and the sound-induced flash illusion). We also used a standard speech-in-noise paradigm to assess individuals' speech perception abilities. Finally, real world measures of socio-communicative abilities were measured using the ADOS-2 and the Social Responsiveness Scale. Behaviourally, perceptual binding at all levels were related to communicative abilities, and differently so for individuals with ASD relative to their typically developed peers. Individuals with ASD showed strong, positive relationships between their abilities to perceptually bind across sensory modalities and to perceive spoken language. At the more local level, visual binding was inversely related to speech perception abilities, suggesting a trade-off in ASD between more global, between-sensory perception and more local, within-sensory perception. These data converge with fMRI data suggesting that individuals with ASD are less able to make use of the sensory cues to perceptually bind across sensory modalities, which leads to decreases in neural processing efficiency with socio-linguistic, audiovisual stimuli. Furthermore, this evidence that higher-order cognitive function in ASD, in this case speech perception, is strongly related to lower-level perceptual differences, suggest that early sensory processing may be a valuable target for future remediation strategies in ASD.

Disclosures: R.A. Stevenson: None. **M. Segers:** None. **M.D. Barense:** None. **S. Ferber:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.02/E42

Topic: C.06. Developmental Disorders

Support: NJ Governor's Council for Autism Research and Treatments

The Nancy Lurie Marks Family Foundation Early Career Award

Title: Characterization of levels of corporeal awareness in Autism Spectrum Disorders and neurotypical controls

Authors: *V. KALAMPRATSIDOU[1], E. B. TORRES[2];
[2]Psychology Dept., [1]Rutgers Univ., Piscataway, NJ

Abstract: This work tests the extent to which it is possible to detect and improve corporeal self-awareness by combining a variety of external sensory inputs with kinesthetic re-afference from the person's real-time movements. In previous work we developed a paradigm that permits probing the person's ability of distinguishing self from others. That paradigm presented the person with a movie of an avatar endowed with veridical motions of the person and with noisy variants of it. A decision making task determining 'ME' vs. 'NotME' was used to assess the person's ability to recognize self-motions. Here, upon initial testing to assess the ability to recognize self-motions, we have the participant physically interact with the avatar as we steer the kinesthetic re-afferent noise in various ways and then retest the person using the same above-mentioned paradigm. To this end we have developed a co-adaptive interface that projects on a large screen an avatar endowed with the real-time captured motions of the participant using the Phase Space (480 Hz). We first parameterize the stochastic signatures of the motor output variability inherently present in the person's motions during the performance of dancing routines. We use the continuous Gamma family of probability distributions to empirically estimate the stochastic trajectories of 22 joints of the body as they rotate and translate. We then utilize the Gamma shape and scale parameters to generate various sources of noise in the joint angular rotations and their temporal dynamics. We use self-noise and others-noise to selectively alter the original stochastic signatures of the person's motions in segments of the routine performed largely beneath the subjects' awareness vs. deliberate segments. We discuss our results in the context of profiling corporeal awareness in participants in the spectrum of autism vs. neurotypical controls in order to develop proper volitional control, enhance intentionality in the person's actions and probe the extent to which the person learns to distinguish self from others.

Disclosures: V. Kalampratsidou: None. **E.B. Torres:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NJ Governor's Council for Autism Research and Treatments, The Nancy Lurie Marks Family Foundation Early Career Award.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.03/E43

Topic: C.06. Developmental Disorders

Support: NCRR Grant 8-P40 OD012217-25

Title: SHANK3 single nucleotide variant associated with social behavior in rhesus macaques

Authors: *S. MADLON-KAY[1], A. BEY[1], R. PASSMAN[1], L. BRENT[2], K. WATSON[3], P. SKENE[1], J. HORVATH[4], M. PLATT[1], Y.-H. JIANG[1];
[1]Duke Univ., Durham, NC; [2]Univ. of Exeter, Exeter, United Kingdom; [3]Univ. of Colorado, Boulder, CO; [4]North Carolina Central Univ., Durham, NC

Abstract: Autism spectrum disorders (ASD) are characterized by impairments in communication and social interaction that arise from the interaction of genes and experience during development. Mutations in the SHANK3 gene have been identified as one of several monogenic causes of ASD. The functional role of SHANK3 in neural development and function has been illuminated by studies in transgenic mice and flies. However, the role of SHANK3 in the social phenotype of ASD has proven more difficult to decipher, in part due to the relative simplicity of mouse and fly social behavior and the relatively impoverished nature of laboratory social environments. An alternative approach is to study naturally occurring genetic variation in free-ranging populations of animals with complex, human-like social behaviors and environments. Rhesus macaques provide a candidate model species due to their extensive use in both laboratory and field research, homologous neural circuitry, complex social behaviour, hierarchical social structure, and the strong contribution of social competence to biological success. Moreover, prior studies have demonstrated a strong genetic component to social function in these animals. Here, we identify SHANK3 sequence variants in a large free-ranging population of rhesus macaques on Cayo Santiago island and the social phenotypes they predict. We sequenced the SHANK3 region in an initial sample of 285 rhesus macaques. We identified one C>T single nucleotide variant (SNV) in a conserved proline rich region that contains Homer, Dynamin, and Cortactin binding sites. This SNV is non-synonymous, with the major allele coding for Proline (CCT) and the minor allele coding for Leucine (CTT). Forty animals were heterozygous at this allele. We investigated the impact of this SNV on social phenotypes using social network analysis, which quantifies position of each animal in the social network and describes their relationship to the rest of the population. These analyses were based on 2 years of observational data regarding the types and frequencies of interactions occurring between animals in this population. We found that animals heterozygous at this locus had stronger affiliative "betweenness", which is a social metric quantifying an animal's tendency to affiliate with groups of animals that do not affiliate with each other and thus connect otherwise separate social

clusters. We speculate that this SHANK3 SNV may increase social 'adventurousness', thereby increasing willingness to interact with partners outside of one's preexisting social circles. Further functional study may provide additional insight for functional significance of this SNV.

Disclosures: S. Madlon-Kay: None. **A. Bey:** None. **R. Passman:** None. **L. Brent:** None. **K. Watson:** None. **P. Skene:** None. **J. Horvath:** None. **M. Platt:** None. **Y. Jiang:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.04/E44

Topic: C.06. Developmental Disorders

Support: NIH Grant HHSN271201200005I

Title: Altered brain connectivity patterns in face processing in autism spectrum disorder

Authors: *I. M.REZAZADEH[1], S. K. LOO[1], S. J. WEBB[2], M. C. GRABB[3], B. H. KING[2], J. T. MCCRACKEN[1];
[1]UCLA, Los Angeles, CA; [2]Seattle Children's Hosp., Seattle, WA; [3]NIMH, Bethesda, MD

Abstract: Background. Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social cognition, with one manifestation being deficient face processing. Recent studies have found widespread cortical under-connectivity, local over-connectivity, and mixed results suggesting disrupted brain connectivity as a potential neural signature of autism. In the present study, we investigated whole brain connectivity during different stages of face processing using high-density electroencephalography (EEG) in young adults with ASD and compared them with typically developing (TD) controls. Methods. Data: 63 participants (35 with ASD, 28 TD controls), aged 18 to 35 years, participated in this study. They performed the FACES task (Webb, 2012) in which upright and inverted pictures of human faces and objects (houses) were randomly presented while 128-channel EEG was recorded. Behavioral Measures: The Social Responsiveness Scale (SRS) and Autism Diagnostic Observation Schedule (ADOS) scores were collected as measures of social cognition in TD and ASD groups. EEG Pre-Processing: Data were exported to EEGLAB in Matlab for pre-processing. EEG connectivity analyses: A neural connectivity matrix for all possible pairs of electrodes was calculated by measuring coherence values in each 100ms window. Network connectivity patterns (especially in face-sensitive brain regions) were then extracted to measure small-world properties such as network efficiency, clustering coefficient, and path length. Results. Preliminary results suggest significant group differences in cortical activation and brain connectivity patterns in the early and mid-stages of face processing between ASD and controls. Specifically, the ASD group exhibited attenuated cortical activation (i.e., less theta/alpha/gamma power synchronization and

beta-band desynchronization, all p's<0.05) relative to controls. In addition, the ASD group demonstrated higher coherence values suggesting stronger local connectivity (all p's < 0.01). Conclusion. Group differences in neural connectivity patterns between ASD and controls may reflect 1) compensatory mechanisms to overcome deficits such as attenuated cortical activation of individual brain regions; and/or 2) potential neural differences underlying social cognition and behaviors. Reference(s). Webb SJ, Merkle K, Murias M, Richards T, Aylward E, Dawson G. (2012). ERP responses differentiate inverted but not upright face processing in adults with ASD. Soc Cogn Affect Neurosci. 2012 Jun;7(5):578-87

Disclosures: I. M.Rezazadeh: None. **S.K. Loo:** None. **S.J. Webb:** None. **M.C. Grabb:** None. **B.H. King:** None. **J.T. McCracken:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.05/E45

Topic: C.06. Developmental Disorders

Support: NJ Governor's Council for Autism Research and Treatments

The Nancy Lurie Marks Family Foundation Early Career Award

Title: Objective characterization of sensory-motor physiology underlying dyadic interactions during the Autism Diagnostic Observation Schedule-2: implications for research and clinical diagnosis

Authors: *C. WHYATT[1], A. MARS[1], E. M. DICICCO-BLOOM[2], E. B. TORRES[1]; [1]Rutgers Univ., Piscataway, NJ; [2]Robert Wood Johnson Med. Group, New Brunswick, NJ

Abstract: Traditionally viewed as a social-cognitive disorder, Autism Spectrum Disorder (ASD), is conceptualized through the use of a 'triad of impairments': socialization, communication and imagination (1). Current diagnostic tools reflect this overarching bias, focusing on levels of socialization and higher-order thinking posed from one single perspective: that of the examiner. Yet by definition, social interactions require more than one participant. Here we investigate the impact of the closed-loop dyadic interaction on the scores that determine the cut-off of an autism diagnosis. To this end we characterize the statistical signatures of sensory-motor physiological parameters underlying the social exchange between both individuals -the examiner and the examinee- partaking in the behaviors evoked by the social presses of the Autism Diagnostic Observation Schedule-2 (ADOS-2, 2). This standard subjective tool is amenable to systematically assess the dyad under various conditions: (a) same examiner and same child using different modules; (b) different examiners and same child using the same modules as in (a). We report the results of this systematic examination from 20 children with

ASD and 10 age- and sex-matched controls. Preliminary analyses in 10 children reveals that the prompting style of the examiner highly impacts the child's responses and that in turn, the child's response can reshape the style of the same examiner. This work highlights the role of both participants on the ensuing outcome. Results therefore underscore the need to reconsider the design of such diagnostic tools in order to effectively capture the social abilities of the child. More importantly, such tools may reveal the potential capabilities and the types of accommodations and supports that the child may need to better function and adapt within society. We report that from the perspective of a neutral observer, i.e. the objective biometrics used to quantify the outcome of the interaction, these types of current diagnostic tools may be confounded, and statistically falsifiable. Additionally, results illustrate potential implications for research that relies upon such tools in their current form. References 1.Wing, L and Gould, J (1979) Severe impairments of social interaction and associated abnormalities in children: epidemiology and classification, Journal of Autism and Developmental Disorders, 9 (1), 11-29. 2.Lord, C., Rutter, M., DiLavore, P.C., Risi, S., Gotham, K., & Bishop, S.L. (2012). Autism diagnostic observation schedule, second edition (ADOS-2) (Part I): Modules 1-4 [Manual]. Torrance, CA: Western Psychological Services.

Disclosures: **C. Whyatt:** None. **A. Mars:** None. **E.M. DiCicco-Bloom:** None. **E.B. Torres:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.06/E46

Topic: C.06. Developmental Disorders

Support: CONACYT GRANT 287846

Title: Quantitative and qualitative improvement of cognitive and social skills in autistic children after virtual stimulation

Authors: *C. CRESPO-CORTES[1], P. CARRILLO[2], G. A. CORIA-AVILA[3], L. I. GARCIA[3], R. TOLEDO[3], M. E. HERNANDEZ[3], J. MANZO[3];
[1]Ctr. de Investigaciones Cerebrales, [2]Univ. Veracruzana, Veracruz, Mexico; [3]Ctr. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico

Abstract: Previous studies showed benefits of virtual stimulation with the Nintendo Wii console and the "Wii Sports" videogame on motor skills of autistic children. Now we continued to a socialization phase of the same 10 autistic children from 5 to 13 years old, patients from the Child Rehabilitation Center of Veracruz. Again, we used the Nintendo Wii and the "Wii Sports" videogame. Socialization last 15 weeks, playing twice a week, 20 minutes per session. On the

first test children played against the facilitator. On the second, children were exposed to another children from the experimental group. Results from the first test showed that children were capable to identify sports, were interested on facilitator´s game, and evoked communicative intention. 70% or more were capable to recognize their turn, respect the turn and respond the question who´s next? For the second test, results showed 85% of the children displayed similar behaviors, socialize with the partner, and pay attention to the entire game. Thus, we hypothesized that virtual stimulation can improve not only motor skills but also cognitive processes such as memory, attention and learning, and facilitate social behavior. Finally after 16 months of continuous playing, we also hypothesized that they could modify their behavior in other situations, such as home or school. Hence, a group of data was collected from each parent with a survey form, answering daily situations of 3 common areas: school, house and free time. Results showed that 100% of parents have a positive perception about the impact of virtual stimulation on their children. All parents conclude that in addition to other therapies, following the videogame trials children improved faster; parents are sure that this kind of stimulation help to focus the attention of their children at home and at school; 8 of 10 parents perceived improvement in longer periods of waiting time at home (wait for meals, wait when parents are at phone/bathroom/kitchen), on daily situations (stay in line on supermarket) and social situations (tolerate sounds or touch from relatives or friends). They also perceived virtual stimulations trough videogames as a key factor of improvement skills at school, such as coloring, better use of pencils, stay sit and motor skills at gymnastics. Thus, we suggest that long-term virtual stimulation could be used as a good tool for the behavioral and cognitive improvement of autistic children.

Disclosures: **C. Crespo-Cortes:** None. **P. Carrillo:** None. **G.A. Coria-Avila:** None. **L.I. Garcia:** None. **R. Toledo:** None. **M.E. Hernandez:** None. **J. Manzo:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.07/E47

Topic: C.06. Developmental Disorders

Support: NIH Grant ZIAMH002920

NIH Grant P30HD040677

Isadore and Bertha Gudelsky Family Foundation

Title: Sex differences in real-world executive functioning in children with Autism Spectrum Disorder

Authors: *E. I. WHITE[1], G. L. WALLACE[2], A. B. RATTO[3], A. C. ARMOUR[3], H. S. POPAL[1], A. MARTIN[1], L. KENWORTHY[3];
[1]NIH, Bethesda, MD; [2]George Washington Univ., Washington, DC; [3]Children's Natl. Hlth. Syst., Washington, DC

Abstract: Females with Autism Spectrum Disorder (ASD) are diagnosed less frequently and later, on average, than males. Questions surround whether these differences could be due to a distinctive profile of behavioral deficits for females with ASD as compared to males. The present study is the largest to date examining executive function and adaptive ability in females with ASD. It utilizes parent ratings of real-world executive functioning and adaptive behavior on the Behavior Rating Inventory of Executive Function (BRIEF) and Vineland Adaptive Behavior Scales-II (VABS-II), respectively, within a well-characterized age and IQ matched sample of 51 males (mean age = 9.94, *SD* = 1.79; mean IQ = 107.22, *SD* = 21.52) and 51 females (mean age = 9.79, *SD* = 1.73; mean IQ = 105.92, *SD* = 21.11) with ASD. A mixed-model ANOVA with Sex (male, female) as the between subjects factor and BRIEF domain (8 scales) as the within subjects factor revealed a main effect of sex (F=11.39; p=.001) which was qualified by an interaction between Sex and BRIEF domain. Post-hoc *t*-tests revealed significantly greater impairments (i.e., higher T scores) for females with ASD on 5/8 BRIEF scales: Inhibit, Initiate, Working Memory, Plan/Organize, and Monitor. These differences appear despite no significant sex differences on the VABS-II and similar correlations between the BRIEF and VABS-II for both males and females. The correlational analyses show that for both males and females with ASD, greater overall executive function impairments (utilizing the Global Executive Composite [GEC] scale of the BRIEF), are related to decreases in Daily Living Skills (males: r=-.35, p<.05; females: r=-.33, p<.05). These results indicate relative executive function weaknesses in females with ASD that occur despite similar adaptive abilities and intelligence and suggest a potentially altered behavioral profile for females, as compared to males, with ASD.

Disclosures: E.I. White: None. **G.L. Wallace:** None. **A.B. Ratto:** None. **A.C. Armour:** None. **H.S. Popal:** None. **A. Martin:** None. **L. Kenworthy:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Psychological Assessment Resources.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.08/E48

Topic: C.06. Developmental Disorders

Title: A new vasopressin V1a antagonist restores normal cognitive and social behavior while revealing a specific brain network in the rat valproate model of autism

Authors: *C. GRUNDSCHOBER, T. MUEGGLER, F. KNOFLACH, C. RISTERUCCI, P. SCHNIDER, B. BIEMANS;
Roche Innovation Ctr., Basel, Switzerland

Abstract: The neuropeptide vasopressin plays an important role in regulating social behavior. In humans, prenatal exposure to the anticonvulsant drug valproate (VPA) has been associated with an increased risk of autism in the newborn. In rats, a single injection of valproate to pregnant dams at day 12.5 of gestation, the time of the neural tube closure, induces a range of behavioral abnormalities in the offspring, such as deficits in social behavior as well as in working and spatial memory. Based on synaptic and phenotypic similarities, the rat VPA model can be considered a valid model of human autism. In this work we investigated the role of central vasopressin 1a (V1a) receptor signaling on the phenotype of the rat valproate model of autism. Rats prenatally exposed to VPA were treated daily during 1 week with a new brain penetrant V1a receptor-specific small molecule antagonist starting at postnatal day 23 or 53. Rat behavior was assessed in the Morris water-maze and in the 3-chamber social interaction test. Long term potentiation was measured in hippocampal slices. Finally, VPA rats and wild-type controls were scanned by functional magnetic resonance imaging at postnatal day 60 and after chronic V1a antagonist treatment, to reveal changes in brain perfusion due to prenatal exposure to VPA and potential normalization by V1a antagonism. At postnatal day 60, chronic treatment with our V1a receptor-specific small molecule antagonist completely reversed the impairments in social behavior, spatial memory and learning typically seen in VPA rats. At postnatal day 30 the deficit in juvenile play behavior was also rescued by antagonist treatment. In line with the behavioral finding, the hippocampal LTP deficit seen in VPA rats was normalized by the compound. In functional magnetic resonance imaging VPA rats were found to be characterized by decreased brain perfusion in piriform cortex, ventral hippocampus and PAG and increased brain perfusion in VTA, dorsal striatum, cingulate and dorsal hippocampus compared to control rats. Chronic V1a antagonism specifically normalized brain perfusion in dorsal striatum, VTA and piriform cortex. Our data show that chronic inhibition of vasopressin V1a receptors restores normal behavior in VPA rats by normalizing perfusion in a brain network important for olfactory processing, repetitive behavior and reward. These results suggest that V1a antagonists have the potential to improve social interaction in autism, a core symptom for which there is currently no drug treatment.

Disclosures: C. Grundschober: A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **T. Mueggler:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **F. Knoflach:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **C. Risterucci:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **P. Schnider:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **B. Biemans:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.09/F1

Topic: C.06. Developmental Disorders

Support: NJ Governor's Council for Autism Research and Treatments

The Nancy Lurie Marks Family Foundation Early Career Award

Title: Characterization of sensory-motor physiological signatures underlying decision making about corporeal self-awareness

Authors: *S. MISTRY[1], P. YANOVICH[2], E. TORRES[3];
[1]Mathematics, Rutgers Univ., Piscataway, NJ; [2]Computer Sci., [3]Psychology, Rutgers, Piscataway, NJ

Abstract: Corporeal self-awareness is fundamental for the development of proper frame of references to be used as anchors and help interpret relative motions within the social scene. Various disorders of the nervous system are currently characterized based on social impairments, but no connection exists between bodily rhythms, their underlying physiology and social interactions. Studies of human motion perception could help us understand this connection but they are often conceived as a top down process. Namely, while we view others in three dimensions, we never see our full bodies in three dimensions. Current approaches cannot explain how humans are able to integrate visual information with the physical body-action component. Here we present a new paradigm for studying biological motions from a bottom up approach with millisecond time precision. We examine the contributions of the peripheral nervous system to the development of volitional control of our decisions through our actions. We ask if there is a congruent map between kinesthetic reafferent signals from our own physiological motions and cognitive social decisions. First we recorded the motions of 16 subjects throughout the body at 15 joint locations performing gait patterns and various exercise routines. Using noise pattern extraction procedures in the temporal and spatial domain, we generated an avatar endowed with the subject's movements. In a perceptual task, we asked subjects to decide "ME" or "NOT ME" and found that despite the noise, subjects could systematically discriminate between the two. Additionally, we uncovered power laws linking velocity dependent parameters in their decisions, body motions, and decision times. Hand speeds were found to be the most predictive and reliable measures while the response times were the most variable. We then endowed stick figures, skeletons, and humanoid avatars with the veridical motions of the subject's decision making to assess the extent to which subjects could recognize themselves making the decision. We registered motion, temperature, heart rate, and galvanic skin conductance to further characterize the physiological signatures of decision-making involving different virtual humans. We report our results in 10 additional subjects.

Disclosures: S. Mistry: None. **P. Yanovich:** None. **E. Torres:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NJ Governor's Council for

Autism Research and Treatments, The Nancy Lurie Marks Family Foundation Early Career Award.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.10/F2

Topic: C.06. Developmental Disorders

Support: SFARI 206683

Autism Speaks Pilot Project Grant 2487

Title: Increased social behaviors and GluA2 phosphorylation in mice-lacking glutamate receptor interacting proteins

Authors: *M. HAN[1], R. MEJIAS-ESTEVEZ[1], R. ROSE[1], S.-L. CHIU[2], A. ADAMCZYK[1], R. HUGANIR[2], T. WANG[1];
[1]Sch. of Medicine, Inst. of Genet. Med., [2]Sch. of Medicine, Dept. of Neurosci., The Johns Hopkins Univ., Baltimore, MD

Abstract: Glutamate receptor interacting protein 1 and 2 (GRIP1/2) are highly homologous neural-enriched scaffolding proteins that bind to the C-termini of glutamate receptors 2/3 (GluA2/3) via their PDZ domains 4-6 (PDZ4-6). GRIP1/2 play an important role in the regulation of GluA2/3 trafficking and neural plasticity. Loss of GRIP1/2 results in decelerated recycling of GluA2 in primary neurons and lack of LTD expression at cerebellar Purkinje cells. Autism-associated gain-of-function mutations of GRIP1 correlate with an accelerated recycling of GluA2 and increased deficits in the reciprocal social interactions in patients. To understand the role of GRIP1/2 in social function, we investigated social behaviors and glutamate-signaling proteins in several brain regions of neuron-specific Grip1/2 double knockout (DKO) mice. DKO mice were generated by crossing Grip2 conventional KO mice with Grip1 conditional (neuron-specific deletion via nestin-cre expression) KO mice and were matched for age, sex, and strain background with wild type (wt) control mice for behavioral testing. Compared to wt mice, DKO mice show a significant increase in sociability, preference for social novelty, and dyadic male-male social interactions. Immunoblot analyses of AMPA type glutamate receptors, metabotropic glutamate receptor mGluR, mTOR, and GABA signaling proteins in DKO mice identified a significant increase in the phosphorylated GluA2 at serine 880 in brain cortex and cerebellum but not striatum. GluA2 phosphorylation is known to affect its interactions with several scaffolding proteins including GRIP1 and PICK1 and alters receptor recycling and AMPA synaptic strength. These data suggest that AMPAR mediated-glutamate signaling in specific brain regions play an important role in the modulation of social behaviors in mice.

Disclosures: **M. Han:** None. **R. Mejias-Estevez:** None. **R. Rose:** None. **S. Chiu:** None. **A. Adamczyk:** None. **R. Huganir:** None. **T. Wang:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.11/F3

Topic: C.06. Developmental Disorders

Title: A direct GABAergic output form the striatum to the amygdala

Authors: *Y. ZHANG[1], X. LI[2];
[1]Dept. of Neurobio., Zhejiang Univ. Sch. of Med., Zhejiang Province, China; [2]Zhejiang Univ., Hangzhou Zhejiang, China

Abstract: The ability to transform stimuli predicting negative outcomes is critical for survival, and perturbations of emotional processing underlie many psychiatric disease states. Classical work focused on the amygdala as a central structure for fear memory. Recent advances, however, have identified the striatum mediating fear learning and the expression of fear behaviors. So, whether these two brain regions have any connections to modulate this fundamental behavior? Here we show that MSNs in the striatum can project to the amygdala. Using the technic of retro tracing, we find that, rather than interneurons expressing parvalbumin (PV), somatostatin (SOM) interneurons receive most innervations from the straitum. More work is still needed to clarify the function of this connection.

Disclosures: **Y. Zhang:** None. **X. Li:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.12/F4

Topic: C.06. Developmental Disorders

Title: Hearing one's name in autism spectrum disorder: an fMRI investigation

Authors: *S. HUEMER[1], F. KRUGGEL[2], V. MANN[3], J.-G. GEHRICKE[4];
[1]Psychology, Loyola Marymount Univ., Los Angeles, CA; [2]Biomed. Engin., [3]Cognitive Sci., [4]Pediatrics, Univ. of California, Irvine, Irvine, CA

Abstract: The goal of this preliminary passive listening study was to compare brain activation between a group of 10 adolescents with autism spectrum disorder (ASD) and 10 neurotypical controls as they passively listened to their own first names. T1 and T2 weighted MR images were acquired on a Philips Achieva 3T scanner, equipped with an 8-channel phased array coil. During each of the three functional imaging sequences, 110 volumes were taken. Besides the images, four contrasts were acquired (SFN=subject's first name, OFN=other familiar (people's) names, OBJ=object of high interest, NUM=number between 1 and 5). General activation patterns were more pronounced when the scores from a test of verbal receptive ability (PPVT) were used (high scoring group = HS, low scoring group = LS) in place of group designation, which supports our theory of distinct activation differences between high functioning and low functioning individuals with ASD that align with verbal ability. When hearing their own name, controls and the HS group continued the pattern of activation in areas of self-referential processing and areas associated with hearing one's name (including BA 7, BA 9, BA 13, BA 17, BA 22, BA 31, BA 19), which we had predicted. Interestingly, we found an involvement of the right hippocampus (BA 54) in the HS group. When hearing their own name, the ASD group showed overall less distributed activation and they relied more heavily on prefrontal structures (especially the frontal pole, BA 10), as we had predicted and as also show activated when the 4-year-old girl with ASD heard her own name in a previous case study (Carmody et al., 2007). Interestingly, we also found increased activation in the left thalamus in the LS group. In summary, subjects who scored higher on the test of verbal ability, the self-referent stimuli activated key brain regions in controls that are linked to self-reference and embedded in long-term memory in a generally more posterior neural network. In comparison, subjects who scored lower on verbal ability activated more anterior brain regions associated with short-term episodic memory. Our study may imply that reduced self-reference is not implicitly "known" but rather acquired and "remembered" like factual information, especially in 'lower functioning' ASD subjects characterized by lower verbal ability but more data is needed to draw any further conclusions.

Disclosures: **S. Huemer:** A. Employment/Salary (full or part-time):; Loyola Marymount University. **F. Kruggel:** None. **V. Mann:** None. **J. Gehricke:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.13/F5

Topic: C.06. Developmental Disorders

Support: W.M. Keck Foundation Postdoctoral Fellowship in Molecular Medicine

Hope Center for Neurological Disorders

Departments of Psychiatry and Genetics at Washington University School of Medicine in St. Louis

NIH Grant R00 NS067239-05

Title: Modeling increased autism risk following maternal SSRI use

Authors: *S. E. MALONEY, S. AKULA, K. CHANDLER, J. D. DOUGHERTY;
Genet., Washington Univ. Med. Sch., Saint Louis, MO

Abstract: The steady increase in prevalence of autism spectrum disorder (ASD) in recent years implies environmental factors in the risk of developing ASD. Four recent studies have reported an increased risk of ASD diagnosis and autistic symptoms in the offspring of mothers taking selective serotonin reuptake inhibitors (SSRIs) during pregnancy, independent of the mothers' depressive symptoms. In addition, transcriptomic analyses of autistic human brains revealed abnormalities in cortical cell-type patterning, implicating transcriptional dysregulation as an underlying mechanism of dysfunction in ASD. The objective of this study was to model the recent human patient population findings to directly examine the risk of presenting ASD-relevant behaviors following developmental SSRI exposure and to understand its consequences on long-term transcription in the brain. Female mice were treated with the SSRI fluoxetine (Prozac; FLX), at the equivalent of the maximum recommended human dose, from just prior to pregnancy through lactation, thus exposing mouse pups to maternal SSRI through neurodevelopment. Behaviors relevant to ASD were assessed in FLX- and vehicle-exposed male and female offspring beginning postnatal and continuing through adulthood. FLX-exposed pups produced fewer ultrasonic vocalizations as compared to vehicle-exposed littermates, indicating the disruption of a behavioral marker of development that is implicated in communication. As adults, the behavior of the mice was also evaluated in behavioral paradigms of sociability, olfactory communication, sensorimotor and locomotor abilities, and repetitive patterns of behavior. To identify transcriptomic consequences of maternal SSRI exposure during neurodevelopment, translating ribosome affinity purification (TRAP) was used to selectively profile actively translated mRNA in a cell-specific manner. RT-qPCR was used to evaluate FLX-induced transcript expression differences in 5HT receptors, trophic factors, excitatory and inhibitory neuron markers and glial markers. This study tests the hypothesis that brain cells may be permanently altered as a result of developmental SSRI exposure, and has the potential to identify persistent molecular changes that may represent targets for treatment. Further, the impact of maternal SSRI use on the risk of developing ASD-related behavioral deficits will be defined. An understanding of the risks of SSRI exposure on the developing brain of a model organism will provide critical information that may have implications for the safety of antidepressant use in pregnant women.

Disclosures: S.E. Maloney: None. **S. Akula:** None. **K. Chandler:** None. **J.D. Dougherty:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.14/F6

Topic: C.06. Developmental Disorders

Support: Roche Postdoc Fellowship Program

Swiss National Science Foundation

Title: Optogenetic and electrophysiological dissection of oxytocin in brain circuits underlying social and fear behavior: differentiating roles for the central and medial amygdala

Authors: C. HEGOUBURU[1], S. GHOSH[1], S. CHENAUX[1], R. TRIANA DEL RIO[1], G. GIOBELLINA[1], I. SALGADO[1], C. GRUNDSCHOBER[2], *R. STOOP[1];
[1]Ctr. For Psychiatric Neuroscience, Univ. Lausanne, Prilly, Lausanne, Switzerland; [2]Roche Pharmaceut. Res. and Early Development, Neurosci. Discovery, Roche Innovation Ctr., Basel, Switzerland

Abstract: The neuropeptide oxytocin (OT) appears to play an important role in social behavior but can also reduce fear responses. OT receptors (OTR) are both found in the medial (MeA) and central amygdala (CeA) where an extensive network of OT fibers that originates from different nuclei in the hypothalamus can be found. Previous works have shown that OT can reduce fear through effects in the CeA, whereas social recognition memory seems to be mediated by the MeA. In the present study we examined to what extent both systems can operate in separate or concerted manner. To compare sensitivity of both nuclei to OT we measured increases in spiking activity in *in vitro* recordings following repeated applications of different OT agonists and blocking effects OTR antagonist. We found responses in 29% of neurons in the CeA and in 18% of neurons in the MeA. Neurons in the MeA exhibited significantly less desensitization upon consecutive OT applications which could be selectively blocked by specific OTR antagonists. To compare contributions of OT signaling in the MeA and CeA in behaving rats, we injected the OTR agonist TGOT and antagonist atosiban targeted through cannulae to either nuclei. OT in the MeA increased sniffing of the companion rat, in both CeA and MeA increased 50 kHz social calls and atosiban in the CeA increased 22 kHz alarm calls. In a custom designed two chamber interaction test, OT in the MeA increased time in proximity to the companion whereas atosiban in both CeA and MeA decreased this interaction time. In a third experiment we assessed interactions between OT signaling in MeA and CeA by measuring effects of social interaction in the two chamber on fear behavior. We found a decrease in fear expression in the presence of the companion rat that was blocked by OT antagonist in the CeA. This could be mimicked by optogenetic stimulation (OpS) of ChR2-OxT neurons in the paraventricular nucleus of the hypothalamus (PVN). Concomitant electrophysiological recordings during exposure to fear revealed rapid increases in neuronal activity of OTergic neurons in the PVN and decreases or

increases in activity in the CeA depending on sensitivity to OT as assessed by OpS. These findings open up the possibility to modulate OT signaling selectively in different brain regions by targeted pharmacological intervention. In addition, they show an interaction between social behavior and fear responses that appears to be mediated by OT in the amygdala. We are currently examining the precise interaction between MeA and CeA in these effects. Our findings may have important implications for the understanding and possible treatment of changes in social behavior among which for example autism spectrum disorders.

Disclosures: **C. Hegouburu:** None. **S. Ghosh:** None. **S. Chenaux:** None. **R. Triana del Rio:** None. **G. Giobellina:** None. **I. Salgado:** None. **C. Grundschober:** None. **R. Stoop:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.15/F7

Topic: C.06. Developmental Disorders

Support: US DoD AR120065

UCI CART

Title: Potential contributions of GABA-A and alpha7 nicotinic receptors to behavior in the BTBR mouse model of autism

Authors: *R. F. YOSHIMURA, M. B. TRAN, D. J. HOGENKAMP, A. J. DUNNIGAN, T. K. GEE, K. W. GEE;
Pharmacol., Univ. of California, Irvine, Irvine, CA

Abstract: Autism spectrum disorder (ASD) is characterized by core behavioral symptoms including deficits in social interaction and stereotyped behavior. ASD is often comorbid with epilepsy, cognitive impairment and anxiety. Studies on ASD patients have demonstrated a down-regulation in the enzymatic synthesis of γ-aminobutyric acid-A ($GABA_A$) and a selective reduction in $GABA_A$ receptor ($GABA_AR$) expression. Restoring normal levels of inhibitory neurotransmission could be a promising avenue to treat ASD. There are multiple pathways to approach this deficit in $GABA_AR$ mediated neurotransmission. One is to enhance the function of the $GABA_AR$'s in response to the endogenous release of GABA. Another is to enhance the release of GABA in response to endogenous stimuli. This can potentially be achieved with positive allosteric modulators (PAM's) of α7 nicotinic acetylcholine receptors (nAChR's) on GABAergic interneurons which, in turn, could enhance the release of GABA. In theory, simultaneously modulating the function of $GABA_AR$'s and α7 nAChR's could enhance the cholinergic activation of α7 nAChRs on the interneuron to promote GABA release while augmenting the postsynaptic responses to GABA at the interneuron-pyramidal neuron synapse.

We have tested the $GABA_AR$ PAM, 2-261, and the α7 nAChR PAM, AVL-3288, in both the social approach and self-grooming behavioral paradigms. By testing these distinct mechanisms individually and in combination, we can potentially elucidate their contributions to the behaviors associated with this mouse model of autism. The data from these studies can help guide us towards novel treatments for ASD.

Disclosures: R.F. Yoshimura: None. **M.B. Tran:** None. **D.J. Hogenkamp:** None. **A.J. Dunnigan:** None. **T.K. Gee:** None. **K.W. Gee:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.16/F8

Topic: C.06. Developmental Disorders

Support: São Paulo Research Foundation, FAPESP grant 2012/07007-8

São Paulo Research Foundation, FAPESP thematic grant 2009/51886-3

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES/Prêmio 1029/2014

Title: Prenatal zinc prevents communication and tyrosine hydroxilase impairments in a rat model of autism induced by prenatal lipopolysaccharide

Authors: *T. B. KIRSTEN[1,2], G. P. CHAVES-KIRSTEN[3], C. SCAVONE[3], M. M. BERNARDI[1], L. F. FELÍCIO[2];
[1]Envrn. and Exptl. Pathology, Paulista Univ., Sao Paulo, Brazil; [2]Dept. of Pathology, Sch. of Vet. Medicine, Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Dept. of Pharmacol., Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Previous investigations by our group have shown that prenatal exposure to lipopolysaccharide (LPS), an endotoxin that mimics infection with Gram-negative bacteria, induces autistic-like behavior. No effective treatment yet exists for autism. Therefore, we used our rat model to test a possible treatment for autism. We selected zinc as the prenatal treatment to prevent or ease the impairments induced by LPS because LPS induces hypozincaemia. Communication, which is impaired in autism, was tested in pups by ultrasonic vocalizations. Because we previously had demonstrated that our rat model of autism presented impairments in the dopaminergic system, i.e., decreases striatal dopamine, its metabolites, and tyrosine hydroxylase (TH) levels, we also evaluated TH protein expression in the striatum and substantia nigra of rats prenatally exposed to LPS and zinc via westernblotting. TH is considered a biomarker of dopamine synthesis because is the first enzyme in the dopamine biosynthetic

pathway. Pups that were prenatally exposed to LPS spent longer periods without calling their mothers, and posttreatment with zinc prevented this impairment induced by LPS to the same levels as controls. Prenatal LPS exposure also decreased 30% the TH levels in the striatum compared to the control group, and zinc posttreatment after LPS resulted in values statistically similar to those of control and LPS groups. Substantia nigra analysis revealed no changes between the three groups. These results showed that prenatal LPS exposure impaired communication, and striatal dopaminergic system of juvenile rats. Because the zinc posttreatment revealed similar TH levels as those of the control group, we concluded that zinc treatment may have prevented the striatal dopaminergic impairment. Thus, prenatal zinc prevented communication, and striatal dopaminergic impairments. The present study revealed a potential beneficial effect of prenatal zinc administration for the prevention of autism, with a possible correlation with striatal dopaminergic mechanism.

Disclosures: T.B. Kirsten: None. **G.P. Chaves-Kirsten:** None. **C. Scavone:** None. **M.M. Bernardi:** None. **L.F. Felício:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.17/F9

Topic: C.06. Developmental Disorders

Support: Intramural grant - Philipps-University

Title: Advanced paternal age as a risk factor for autism: Behavioral and morphological alterations in rats and humans

Authors: *R. K. SCHWARTING[1], D. SEFFER[2], A. KRUG[3], J. C. EGGEBRECHT[2], H. RIPPBERGER[2], B. DIETSCHE[3], H. BACKES[3], T. KIRCHER[3], M. WÖHR[2];
[2]Exptl. and Biol. Psychology, [3]Dept. of Psychiatry and Psychotherapy, [1]Philipps-University of Marburg, Marburg, Germany

Abstract: It is widely recognized that advanced maternal age is a risk factor for bearing a child with mental retardation, such as Down syndrome. In contrast, however, few people are aware of the fact that advanced paternal age (APA) constitutes a risk factor for mental illness in offspring as well. Children born to older fathers have an increased risk of developing severe neurodevelopmental disorders, such as autism (ASD) and schizophrenia, as shown in a number of well-conducted epidemiological studies, with some of them even reporting accumulating risk across generations. This is particular relevant as ASD diagnoses have climbed steadily since the 1970s - along with a marked increase in the number of fathers older than 40 years in the past couple of decades. It is estimated that approximately 10% of the increase in ASD diagnoses is

due to APA. To study APA effects on brain and behavior, a large cohort of 670 healthy subjects was investigated with the schizotypal personality questionnaire (SPQ-B) and the NEO-FFI. It was found that APA had linear effects on SPQ-B sum scores and all of its subscales as well as neuroticism after controlling for maternal age, subjects' age, sex and level of education. In addition, APA was linearly correlated with increased grey matter volume in the right parahippocampal cortex and the right inferior frontal cortex in a subsample of 342 subjects, which is in line with reports of increased grey matter volumes in these brain areas in ASD. However, despite the fact that epidemiological studies demonstrated an association between APA and neuropsychiatric disorders, the underlying causality is not yet understood since experimental evidence in humans is not feasible. Therefore, we recently developed a rat model, comparing offspring from young (2 months) and old (12 months) fathers, while maternal age was the same in both conditions (2 months). By means of this comparison, we found that rats from old fathers display behavioral alterations with relevance to all ASD core symptoms, including social communication deficits and impaired reversal learning. This finding indicates that at least part of the APA effects obtained in humans are not due to differences in personality traits or socio-economic status that have been repeatedly reported when comparing young and old fathers. As recently suggested effects might be linked to reduced quality of spermatocytes due to epigenetic modifications or accumulating genetic deficits, i.e. mutations, as a consequence of "copy errors" during cell division. Since there is evidence for altered hippocampal size in ASD diagnosed patients, rats' brain morphology was investigated at different developmental stages.

Disclosures: **R.K. Schwarting:** None. **D. Seffer:** None. **A. Krug:** None. **J.C. Eggebrecht:** None. **H. Rippberger:** None. **B. Dietsche:** None. **H. Backes:** None. **T. Kircher:** None. **M. Wöhr:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.18/F10

Topic: C.06. Developmental Disorders

Support: Wellcome Trust Grant WT802131MF

Medical Research Scotland Grant 206FRG

Shirley Foundation

Dr Mortimer and Theresa Sackler Foundation

Title: Common symptoms, distinct brain function: an fMRI study of social cognition in the autism and schizophrenia spectrums

Authors: *A. STANFIELD[1], R. PHILIP[2], H. WHALLEY[1], L. ROMANIUK[1], J. HALL[3], E. JOHNSTONE[1], S. LAWRIE[1];
[1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Tailor Ed Fndn., Edinburgh, United Kingdom; [3]Univ. of Cardiff, Cardiff, United Kingdom

Abstract: Introduction: Although there are overlaps between autism and schizophrenia, especially in social domains, in their most severe forms they are usually readily distinguished clinically by their age of onset, degree of communication impairment and the presence or absence of positive psychotic symptoms. However, it is now recognized that there exist 'spectrum' forms of both disorders without such marked and distinguishing impairments. Autism spectrum disorders (ASD) and schizophrenia spectrum disorders, such as schizotypal personality disorder (SPD), have many overlapping and few distinguishing features, particularly in social domains. However, it is not known whether these social deficits result from shared or distinct brain mechanisms. To establish this, we compared social cognition in ASD and SPD using functional magnetic resonance imaging (fMRI). Methods 21 individuals with SPD, 28 with ASD, 10 comorbid for both conditions (CM) and 33 controls were compared with respect to clinical symptoms (Positive and Negative Syndrome Scale); neuropsychological measures of social cognition (social judgement and Ekman60 faces tasks); and an fMRI task where they made social or non-social judgements from face stimuli. Results The ASD and SPD groups showed few differences in negative symptoms or social cognition outwith the scanner. However, fMRI showed that, compared to those with ASD, the SPD group showed greater activation increases when making social compared to non-social judgements in three clusters covering posterior cerebellum, fusiform and inferior temporal gyri bilaterally, the left superior temporal sulcus and temporoparietal region, and right occipital regions; further hyper-activation was identified in the SPD group in left amygdala using a small volume correction (all $p<0.05$ family-wise error corrected). Control activations lay between the ASD and SPD groups. CM individuals showed frontal activation differences compared to the ASD group alone. Conclusions Although the social cognitive deficits in ASD and SPD appear superficially similar they are the result of different brain mechanisms. These differences have implications for therapeutic interventions targeted at social dysfunction in these conditions and validate their classification as separate disorders.

Disclosures: A. Stanfield: None. **R. Philip:** None. **H. Whalley:** None. **L. Romaniuk:** None. **J. Hall:** None. **E. Johnstone:** None. **S. Lawrie:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.19/F11

Topic: C.06. Developmental Disorders

Support: Wells Fargo

Title: Audiovisual processing of social robot stimuli: understanding therapeutic outcomes in adolescents with autism spectrum disorder

Authors: *L. F. PRZYBYLOWSKI, III[1], F. SARTORATO[1], A. PHILLIPS[1], M. PROUGH[2], J. J. DIEHL[2], D. K. SARKO[1];
[1]Anat., Edward Via Col. of Osteo. Med., Spartanburg, SC; [2]Psychology, Univ. of Notre Dame, Notre Dame, IN

Abstract: For children with autism spectrum disorder (ASD), social robots are being increasingly utilized as therapeutic tools in order to enhance social skills and communication. Social robots strike an ideal balance between inanimate toys, which do not elicit social behaviors, and human beings, whose body language and social interactions can frustrate and distress individuals with ASD. These robots have been shown to increase engagement and attention in adolescents with ASD, particularly in the age range of 12 to 17. The perceptual mechanism underlying these behavioral benefits remains poorly understood, but may be related to enhanced processing of audiovisual cues during social interactions with robots. To test this, we examined the effect of naturalistic versus robotic audiovisual stimuli on the size of the temporal binding window (a timeframe in which two stimuli from different sensory modalities are likely to be perceptually bound as a unified, synchronous event) in adolescent children. One representative "unit of social interaction" was chosen for the initial stimulus set: videos of naturalistic human speech paired with biological motion (a human saying "no" while shaking her head) versus comparable robotic speech paired with biological motion (a social robot, used clinically, saying "no" while shaking its head). Subject perceptual responses were tested at various stimulus onset asynchronies (SOAs). We predicted that a narrower temporal binding window (indicating enhanced audiovisual integration) would be seen for naturalistic stimuli in healthy subjects, but that robotic speech stimuli might result in enhanced audiovisual processing for subjects with ASD. Although multisensory processing (including audiovisual integration) is known to be impaired in individuals with ASD, we hypothesize that social robots may confer effective therapeutic outcomes through enhanced processing of audiovisual cues, particularly during communication and social interactions. Ultimately, clinical outcomes might be elucidated through assessment of factors such as alleviation of social anxiety or amelioration of hyper/hyposensitivity to various sensory stimuli. Such experiments have the potential to strengthen the evidence supporting the clinical use of robots for individuals with ASD through psychophysical assessment of perceptual outcomes.

Disclosures: L.F. Przybylowski: None. **F. Sartorato:** None. **A. Phillips:** None. **M. Prough:** None. **J.J. Diehl:** None. **D.K. Sarko:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.20/F12

Topic: C.06. Developmental Disorders

Title: Developmental hyperserotonemia affects partner play preference and reduces oxytocin expressing cells in the adult PVN of male, but not female, rats

Authors: *K. WAGNER, A. M. K. MADDEN, S. L. ZUP;
Psychology, Univ. of Massachusetts Boston, Boston, MA

Abstract: There are marked sex differences in human behavior and disease, including a pronounced disproportion in the incidence of neurodevelopmental disorders such as Autism Spectrum Disorder (ASD), with males being diagnosed more often than females. Elevated blood serotonin (hyperserotonemia) in perinatal development (DHS) has been implicated in ASD pathogenesis. Thus, pre-and postnatal administration of a general serotonin agonist, 5-methoxytptamine (5-MT), has been used as a model of DHS to investigate the social behavior and relevant brain morphology implicated in ASD. Our previous study examining the effects of DHS found that male juvenile rats actually played less than females, findings that oppose the accepted dogma that males consistently exhibit more play behavior. We hypothesized that the reduced male play was due to play partner preference changes, such that vehicle males weren't playing less per se, but were avoiding play with 5-MT treated males. To test this possibility, we used a three-chamber sociability partner preference paradigm. On postnatal day 25 (P25) control males preferred to play with vehicle females over vehicle males; however, when forced to choose between vehicle females and 5-MT treated males, this female preference is abolished. These findings suggest that vehicle males can distinguish between other vehicle males and 5-MT treated males. Furthermore, 5-MT treated males do not show the same female preference, demonstrating a marked change from control males and thus possibly an abnormal social behavior profile. In order to determine potential neurochemical correlates to these behavioral changes, we next examined the number of oxytocinergic (OXT+) cells -in the paraventricular nucleus (PVN) in adult vehicle and 5-MT treated male and female rats. Our previous findings have shown that DHS treatment reduces the number of OXT+ cells in the medial PVN of juvenile females, but not males. Interestingly, by adulthood, DHS males have a decreased number of OXT+ compared to control males; however, a possible sex difference in OXT+ in adult animals has never been examined. Current preliminary data demonstrate that adult males treated with 5-MT perinatally have fewer OXT+ cells in the PVN than any other group, including adult females perinatally treated with 5-MT, suggesting that the reduced number of OXT+ cells seen in juvenile females may serve ultimately as a protective mechanism, especially if normal OXT+ cells are restored by adulthood.

Disclosures: K. Wagner: None. **A.M.K. Madden:** None. **S.L. Zup:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.21/F13

Topic: C.06. Developmental Disorders

Support: NJ Governor's Council for Autism Research and Treatments

The Nancy Lurie Marks Family Foundation Early Career Award

Title: A neural correlate of disembodied intention?

Authors: *N. TADIMETI[1], J. COLE[3], E. TORRES[2];
[2]Psychology, [1]Rutgers Univ., Piscataway, NJ; [3]Neurol., Poole Hosp. NHS Fndn., Poole, United Kingdom

Abstract: There are proprioceptive pathways to the posterior parietal cortex (Prevosto et al 2011) that may participate in the formation of intentional plans for action. In the non-human primate model (rhesus macaques), this part of the cortex has been implicated in forward planning (Mulliken and Andersen, 2008) and anticipatory trajectory planning (Torres et al 2013). The question of whether kinesthetic reafference from ongoing movements is necessary to update the forward model and maintain intentional plans is still open. Addressing this question would also shed light on the distinction between embodied cognitive (volitional) control and disembodied intent. For example, work involving stimulation of the parietal and pre-motor regions in human subjects has proposed that in the absence of electromyographic activity from movements, the subjective feeling of motor execution seems to emerge from conscious intention and its predicted consequences, rather than from kinesthetic afference (Desmurget et al., 2009). Yet, these participants had intact sensory afferent fibers. It remains unknown how the brain's networks would be engaged in the absence of sensory kinesthetic afferent input from the body. The present work involving subject IW without afferent input from the neck down (Cole and Sedgwick, 1992), enables us to investigate the question relating kinesthetic reafference and intentionality. Electroencephalographic activity (EEG) using the Active Two multi-channel BioSemi system (Amsterdam, the Netherlands) was registered (scalp signals at 256 Hz sampling rate from 64 channels using the international 10-20 system) as participants attempted to control the left and right directions of an external cursor (Torres and Choi, 2013). Twenty subjects (29years old +/-7) participated including IW, a 61 year old left-handed man who had suffered a peripheral deafferentation at the age of 19 years old. We show the dynamically unfolding network patterns using a combination of stochastic analyses (Torres et al 2013) and the brain connectivity toolbox (Rubinov and Sporns, 2010). We identified self-emerging modules and hubs and characterized the stochastic signatures of the flow between brain regions. We report the extremely different dynamics of IW in relation to controls.

Disclosures: **N. Tadimeti:** None. **J. Cole:** None. **E. Torres:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.22/F14

Topic: C.06. Developmental Disorders

Support: NIH Grant HD05311

NIH Grant MH099660

NARSAD Independent Investigator Award

Grant-in-Aid No. 4501 Japan

NIH Grant DC007690

Title: Structure and function of neonatal social communication in a genetic mouse model of autism

Authors: T. TAKAHASHI[1], S. OKABE[5], P. O'BROIN[2], A. NISHI[1], K. YE[3], M. V. BECKERT[4], T. IZUMI[1], A. MACHIDA[5], G. KANG[1], J. PENA[4], A. GOLDEN[2], T. KIKUSUI[5], *N. HIROI[6];
[1]Dept. Psychiat Behav Scie, [2]Dept. Genet., [3]Dept. Epid. Population Hlth., [4]Dept. Neurosci, Albert Einstein Col. of Med., Bronx, NY; [5]Azabu Univ., Sagamihara, Japan; [6]Psychiatry and Behavioral Sci., Albert Einstein Col. Med., Bronx, NY

Abstract: Babies with incipient autism spectrum disorders (ASDs) and pups of genetic mouse models of ASDs exhibit atypical vocalizations. However, the precise sequence structure of and functional impacts of such atypicalities on social communication between babies and mothers have not been isolated and determined. We used vocal call data from a genetic mouse model of ASDs to test the hypothesis that call type sequences have functional impacts on maternal approach. Calls were recorded from Tbx1 heterozygous and wild-type littermates at P8 or P12 during 5-min maternal separation. Tbx1 heterozygous pups emitted significantly fewer complicated call types, compared to wild-type pups at P8; vocal calls considerably declined thereafter for wild-type pups so that the two groups were indistinguishable for any call type by P12. Wild-type pups emitted longer complicated call types than heterozygous pups at P8. Wild-type pups exhibited decreased lengths of these calls by P12 so that the two genotypes no longer differed at that time. Wild-type and heterozygous pups did not differ in the pitch or peak amplitude of vocal calls. Shannon entropy analysis showed that pups non-randomly chose call types to emit two, three and four successive calls. A sequence structure of calls exists in normal mouse pups and Tbx1 heterozygous pups have a higher degree of non-random sequence. Markov modeling determined the predominant sequences of calls of wild-type and heterozygous pups. Wild-type pups more frequently connected complicated call types than heterozygous pups. In contrast, heterozygous pups more frequently formed connections among simple call types than wild-type pups. A Sparse Partial Least Square Discriminant Analysis (sPLS-DA) showed that

wild-type pup call sequences were more individually variable along the two identified components, compared to call sequences of heterozygous pups. Finally, we tested the effects of wild-type and heterozygous call sequences on maternal approach. We used C57BL/6J mothers 7-8 days postpartum to assess their response to the representative call sequences of Tbx1 wild-type and heterozygous pups. C57BL/6J mothers stayed longer in the tube from which wild-type calls were presented compared to that from which no call was presented; heterozygous calls did not induce such a preference. When a randomized wild-type sequence was presented, mothers did not show a preference for the sound tube compared to the no-sound tube. Our data suggest that an ASD risk gene has a negative impact on social communication with mothers due to atypical sequence structures of vocalizations during the neonatal period.

Disclosures: **T. Takahashi:** None. **S. Okabe:** None. **P. O'Broin:** None. **A. Nishi:** None. **K. Ye:** None. **M.V. Beckert:** None. **T. Izumi:** None. **A. Machida:** None. **G. Kang:** None. **J. Pena:** None. **A. Golden:** None. **T. Kikusui:** None. **N. Hiroi:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.23/F15

Topic: C.06. Developmental Disorders

Support: CONACYT scholarship #281550

Title: Potential N170 in mexican children with autism spectrum disorder

Authors: *C. VELA[1,2], P. TORRES[3], D. E. GRANADOS[4];
[1]Psychobiology laboratory, Univ. Veracruzana, Xalapa-Enriquez, Mexico; [2]Doctorado en Ciencias Biomédicas, [3]Doctorado en Investigaciones Cerebrales, [4]Lab. de Psicobiología, Univ. Veracruzana, Xalapa-Enríquez, Veracruz, Mexico

Abstract: Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder, characterized by impairments in social and comunicative behavior, there are stereotyped behaviors and restricted interests, which may or not be associated with intellectual deficit (APA, 2013). The use of the electroencephalogram (EEG) is a noninvasive method to examine the activation time in which the brain processes sensory and cognitive information, allowing study brain function during normal development and diseases. The event-related potentials (ERP) refer to the average electrical signal recorded in relation to a specific time event (Ahmadlou & Adeli, 2014). Face processing task are usually used for investigation of emotional and social abnormalities of ASD patient. A commonly investigated visual ERP component in face perception is the N170. The N170 has been associated with the perceptual processing of faces. A negative peak occurring approximately about 160-170 ms in response to pictures of faces (Bentin

et al.1996). ASD children have shown prolonged N170 latencies compared with normal children (Hileman et al. 2011). Aim: To describe the response of N170 in perception of face and balloons in children with ASD. Methods: Eight children with ASD (age: 6-13) and eight developmental typical children participed in this study. In children with ASD were observed different severity levels. We recorded full-scalp EEG while the children looked at pictures. The stimuli of neutral unfamiliar faces and balloons were presented in gray-scale images. EEG was recorded using Neuroscan system, with a 64 electrodes. The data were average across three electrodes (O1, Oz, O2) and the time window extending from -100 ms to 500 ms. Results: No significant differences were observed in N170, the response of children with ASD was to 218 ms (-3 μv) for faces and 219 ms (-3.3 μv) for balloons, while the response of children with developmental typical was to 201 ms (-4 μv) for faces and 194 ms for balloons (-4.6 μv). Conclusions: The responses of N170 in faces were later in children with ASD. It is necessary to increase the sample size to confirm the data.

Disclosures: C. Vela: None. **P. Torres:** None. **D.E. Granados:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.24/F16

Topic: C.06. Developmental Disorders

Support: New Jersey Governor's Council for Autism Research and Treatments

The Nancy Lurie Marks Family Foundation Early Career Award

Title: Unifying statistical framework to study brain-body physiological interactions in typical and pathological nervous systems

Authors: *E. B. TORRES;
Psychology Dept, Rutgers Univ., Piscataway, NJ

Abstract: The focus of mental illnesses and neurological disorders has been primarily on the central nervous system (CNS), specifically the brain and the spinal cord. The peripheral nervous systems (PNS) however, are bound to play a fundamental role in providing internal kinesthetic sensory feedback to enable corporeal and facial awareness. These are necessary ingredients to build a frame of reference on the self so as to compute relative relations in the external world, including those occurring in the social scene. Failure to form or to maintain such anchors results in disorders of the nervous system that give rise to social and cognitive impairments. Such impairments manifest in different forms clinically termed autism, schizophrenia, bipolar disorder, executive dysfunction, among others. At present it is unknown what the role of sensory-motor information may be in these disorders, particularly the potential roles of re-

afferent kinesthetic information from mechanoreceptors, nociceptors and thermo-receptors in scaffolding and maintaining social and cognitive abilities. We here present a new statistical framework that enables the combination of various signals from motions and autonomic functions (heart rate, temperature, blood flow volume, etc.) to enable the characterization of various behavioral states in a number of disorders of the nervous system where social interactions are impeded. We provide a statistical parameterization of individuals in the spectrum of autism and schizophrenia in relation to de-afferentation, stroke and Parkinsonism. The same statistical framework used here to study bodily interactions is then used to analyze time-series of EEG signals from Brain Computer Interfaces (BCI) interactions, thus providing a way to connect the rate of change of bodily statistical signals with that of EEG activity. We combine this new statistical platform[1] with tools from the brain connectivity toolbox[2] to analyze brain activity dynamically. We then adapt such tools to dynamically track the peripheral network's activities across the body. We discuss our results across different pathologies and underscore the need to distinguish (and connect) in each case mental intent and physical body volition. **References** 1 Torres EB, Cole J, Poizner H (2014) Frontiers in Human Neuroscience 8:823-19. 2 Rubinov M, Sporns O (2010) NeuroImage 52:1059-69.

Disclosures: E.B. Torres: None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.25/F17

Topic: C.06. Developmental Disorders

Support: SPH student research grant to S.M.Owen

Title: Anodal and cathodal tDCS as a therapy for fine motor skill impairment in Autism Spectrum Disorder

Authors: *S. M. OWEN[1], N. HOSEINI[1], G. C. FREY[1], H. J. BLOCK[1,2];
[1]Kinesiology, [2]Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Restricted, repetitive behaviors (RRBs) are one of the core diagnostic criteria of autism spectrum disorder (ASD) yet there is little information on the causes and treatment of these debilitating symptoms. These behaviors range from simple repetitive motor behaviors to more complex cognitive behaviors such as compulsions and restricted interests. Two distinct types of RRBs have been conceptualized. "Lower order" repetitive sensory and motor behaviors and "higher order" rigid cognitive behaviors. Perry et al. (2007) demonstrated that sensorimotor gaiting and lower order RRBs reflect inhibitory abnormalities. According to the motor control theory, the RRBs common to those with ASD occur as the result of a deficient motor system and

its attempts to maintain homeostasis and engage in goal-oriented motor skills (Radonovich et al., 2013). Impairments in motor control are the most commonly reported research findings associated with ASD. The purpose of this study is to determine whether or not manual dexterity can be improved by manipulating inhibition and excitation of the motor cortex with tDCS. To date, 4 subjects, ages 18-25 years, right handed, and with the clinical diagnosis of ASD, have each completed 3 conditions. Conditions consisted of anodal (excitatory), cathodal (inhibitory), and sham tDCS, experienced in different visits at least 4 days apart. Condition order was randomized across subjects. Each subject was timed as they performed a block of eight baseline trials on a Purdue Pegboard Test with 1 minute rest between trials. The goal was to have subjects plateau so that we could control for any learning effect. Next, each subject received 15 minutes of anodal, cathodal, or sham tDCS. Subjects were given 5 minutes rest when the tDCS was initiated and then continued with their second block of eight pegboard trials for the remaining 10 minutes. The results of the timed trials were then averaged to determine whether stimulus type had any effect on performance and whether performance became faster or slower. Preliminary results for these subjects show that on average, speed increased 2.2% in the sham condition, 4.0% in the anodal condition, and speed decreased 0.4% in the cathodal condition. However, there was considerable inter-subject variability; two subjects showed improved performance with anodal stimulation while the other two did not. These results suggest that application of anodal tDCS may help to improve fine motor skill in this population.

Disclosures: **S.M. Owen:** None. **N. Hoseini:** None. **G.C. Frey:** None. **H.J. Block:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.26/F18

Topic: C.06. Developmental Disorders

Support: The Nancy Lurie Marks Foundation Early Career Award

Title: Characterization of sensory-motor physiological signatures of typical and atypical gait patterns in autism of known and unknown etiology

Authors: ***D. WU**[1], J. NGUYEN[2], S. MISTRY[3], E. TORRES[4], J. V. JOSÉ[1,5];
[1]Physics, Indiana Univ., Bloomington, IN; [2]Cell Biol. and Neurosci., [3]Biomath., [4]Psychology, Rutgers Univ., Newark, NJ; [5]Integrative and Cell. Physiol., Indiana Univ. Med. Sch., Indianapolis, IN

Abstract: Autism Spectrum Disorders (ASD) encompasses a very heterogeneous group of individuals with neurodevelopmental delays and atypical behavioral patterns, often including odd gait. Across the broad spectrum of ASD some subgroups are of idiopathic origins, while in

others the disorders can be traced back to a genetic mutation/deletion. In such cases it is amenable to characterize the sensory-motor physiology underlying various features of the behavioral phenotype, which is currently described by observational methods. One group of interest in ASD is 22q13 Deletion Syndrome (Phelan-McDermid Syndrome, PMS) caused by SHANK3 gene deficiency. The SHANK3 codes for a master scaffolding protein that forms a key framework in the postsynaptic density of glutamatergic synapses. Efficient synaptic transmission at the central and peripheral levels of the nervous system is critical for motor control, so its disruption can interfere with all aspects of behavioral development required for proper social and cognitive interactions. Because PMS gives rise to both sensory-motor issues and an autistic phenotype, it offers a model to help us understand social deficiencies in relation to sensory-motor deficiencies. This study characterizes the sensory-motor physiology underlying gait patterns in children with PMS who also received a diagnosis of ASD. We compare their signatures to those of individuals with idiopathic ASD and with neurotypical controls. In this ongoing study we have characterized thus far the signatures of 15 PMS children of both sexes (ages 6-16 years old) and 10 neurotypical age- and sex-matched controls. Further we have begun the characterization of individuals with idiopathic ASD. We have found a pattern of gait controls that is absent from all PMS participants and present only in 50% of the participants with idiopathic ASD. We discuss our findings in light of new biometrics to characterize both overlapping and disjointed features of PMS-ASD and idiopathic-ASD.

Disclosures: **D. Wu:** None. **J. Nguyen:** None. **S. Mistry:** None. **E. Torres:** None. **J.V. José:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.27/F19

Topic: C.06. Developmental Disorders

Support: New Jersey Governor's Council for Autism Research and Treatments

The Nancy Lurie Marks Family Foundation Early Career Award

Title: Characterization of sensory-motor behavior under different mindsets

Authors: ***J. RYU**, E. TORRES;
Rutgers Univ., Piscataway, NJ

Abstract: Evidence has shown that our motions exhibit different signatures of variability depending on the level of intent (Torres, 2011). The stochastic signatures of speed variability from goal-directed movements differ from those of supplemental movements. This suggests that different principles may govern these different types of movements. However, we do not know

whether such signatures may be affected by an individual's type of mindset. Here we explore how and whether different mindsets lead an individual to exhibit different characterizations of sensory-motor behavior. In the experiment, participants were instructed to touch the screen when prompted. Subsequently, participants heard a high tone that rang 100ms, 400ms, or 700ms after the touch. Participants were then provided with a sliding scale between 0 to 1 second, and were to indicate how long they perceived the time elapsed between the touch and the high tone. They performed this task on 60 trials in three conditions. All participants started with the control condition, where they performed this task right after they became familiarized with the procedure. In the mindfulness condition, participants performed a meditation practice, and then performed the task. In the cognitive load condition, participants were given a dual task to count backwards from 400 by 3 while they performed the task. In order to collect data, motion sensors were attached to the upper body including the index finger to measure the change in position, velocity, and acceleration at each moment. The positional trajectory of the pointing and retracting movements were examined along with the temporal speed profiles of those movements, allowing us to extract and plot a frequency distribution of the kinematic parameters (e.g., peak speed, time to peak speed). We used maximum likelihood estimation to empirically estimate the shape and scale parameters of the continuous Gamma family of probability distributions. We charted each subject on the Gamma parameter plane and tracked the levels of predictability and reliability of their speed levels. Our preliminary data suggests that cognitive overload increases the noise-to-signal ratio and decreases the predictability of the subjects' speed maxima, than under mindful or control condition.

Disclosures: J. Ryu: None. **E. Torres:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.28/F20

Topic: C.06. Developmental Disorders

Support: NJ Governor's Council for Autism Research and Treatments

The Nancy Lurie Marks Family Foundation Early Career Award

Title: Sensory-Motor physiological signatures underlying natural behaviors: A characterization across the human spectrum ranging from typical to pathological states

Authors: *U. V. MAJMUDAR, J. NGUYEN, E. TORRES;
Rutgers Univ., Piscataway, NJ

Abstract: The recent revolution in wearable sensors facilitates the continuous registration of a broad spectrum of motion signals during the performance of natural activities of daily living,

including sleep and exercise. Various aspects of sensory-motor physiology underlying behavioral states can now be objectively characterized with unprecedented precision, beyond verbal reports and subjective inferences. Integrating various outputs from such sensors can give us long time series of kinematics and autonomic-system signals throughout the day and across months of continuous recordings. This on the other hand poses new problems to develop analytics that handle large volumes of rapidly accumulating data. We here present new analytical techniques to handle big data and process various physiological signals such as motion, skin surface temperature, heart rate, blood volume, among other outputs from wearable sensing technology. Subjects performed tasks including dancing, walking, sleeping, decision-making, and tasks in response to perceptual stimuli, while wearing sensors on various parts of the body for up to 15 hours. Inertial measurement units (APDM 128Hz, Oregon) recorded acceleration and gyration. A graphical user interface (GUI) was designed and implemented in MATLAB to automatically process large volumes of data and provide longitudinal tracking of the evolution of the stochastic signatures of the subjects' physiological signals. In addition to the use of the GUI in typical populations, we demonstrate the use of this GUI in a variety of clinical populations with neurological disorders and mental illnesses. The latter include schizophrenia, bipolar disorder, and general executive dysfunction. The former included autism, sensory processing disorder, and a genetic deletion giving rise to an autistic phenotype. We propose the use of the GUI interface and the statistical platform to characterize bodily responses and provide real-time, objective read-outs of the subject's volitional control as a function of statistically predictive outcomes. Furthermore, during therapeutic interventions we use the wearables and biometrics to characterize and track levels of anxiety, stress, dysregulation as well as compliance, self-regulation and positive outcomes. These objective outcome measures provide indexes to assess the effectiveness, risk and generalization of a plethora of interventions that are now subjectively assessed and for which no insurance coverage currently exists.

Disclosures: **U.V. Majmudar:** None. **J. Nguyen:** None. **E. Torres:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.29/F21

Topic: C.06. Developmental Disorders

Support: CONACYT grant 138663

Title: Cerebrolysin remodels neuronal morphology in the limbic system and improves behavioral deficit in rat model of autism

Authors: ***M. E. BRINGAS**[1,2], M. MAXIMINO ROJAS[1], O. APARICIO[1], C. ESCOBAR JARQUÍN[1], S. R. ZAMUDIO HERNÁNDEZ[2], F. DE LA CRUZ[2], M. ATZORI[3,4], G. FLORES[1];

[1]Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Dept. de Fisiologia, Inst. Politecnico Nacional, Mexico, DF, Mexico; [3]Sch. of Behavioral and Brain Sciences, Univ. of Texas at Dallas, Richardson, TX; [4]Facultad de Ciencias, Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico

Abstract: Prenatal valproic acid (VPA) exposure has been proposed as an animal model reproducing both behavioral and anatomical impairments of autism spectrum disorders (ASD). Some of the behavioral features of the model are hyper-responsiveness to novel environment and decreased number of social behaviors. Learning and memory in this model have not been analyzed in detail. Our group has previously reported rearrangement neuronal dendritic in several limbic regions on rats exposed to VPA in gestational stage. There are some studies in which the neurotrophic peptide mixture Cerebrolysin (Cbl) has been used to ameliorate abnormalities in models of aging and neurodevelopmental disorder such as Alzheimer, schizophrenia and brain injury. Three studies of Cbl administration in autistic patients have yielded promising results. In this study we aimed at evaluating the effect of Cbl administration (5 to 21PD) on pups (age 21PD) and adult rats (age 70PD) prenatally exposed to VPA. We determined the effects of Cbl on behavioral response (social interaction, responsive to novel environment), learning and memory (novel object recognition), and neuronal morphology (using Golgi-Cox stain) in prefrontal cortex, amygdala, hippocampus and nucleus accumbens. We found that Cbl administration has a different effect on hyper-responsiveness to novel environment, social interaction and memory; as far as morphology is concerned, we have found interesting changes in dendritic morphology and density spines in all the limbic regions studies after Cbl administration; in all those issues, the results were different depending on the age.

Disclosures: M.E. Bringas: None. **M. Maximino Rojas:** None. **O. Aparicio:** None. **C. Escobar Jarquín:** None. **S.R. Zamudio Hernández:** None. **F. de la Cruz:** None. **M. Atzori:** None. **G. Flores:** None.

Poster

306. Behavioral Analyses in Autism Spectrum Disorder

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 306.30/F22

Topic: C.06. Developmental Disorders

Support: Fonds de la Recherche en Santé du Québec (FRSQ)

Autism Research Training program (ART)

NeuroDevNet doctoral fellowship

Title: An association between lower- and higher-level visual perception in autism spectrum disorder

Authors: *J. GUY[1,2], L. MOTTRON[3], C. BERTHIAUME[3], A. BERTONE[1,2,3];
[1]Integrated Program in Neurosci. (PNLab), McGill Univ., Montreal, QC, Canada; [2]Perceptual Neurosci. Lab. for Autism and Develop., Montreal, QC, Canada; [3]Ctr. d'excellence en Troubles Envahissants du Développement de l'Université de Montréal (CETEDUM), Hôpital Rivière-des-Prairies, Montreal, QC, Canada

Abstract: Individuals with Autism Spectrum Disorder (ASD) present a perceptual profile that is defined by atypical performance on tasks mediated by lower- (primary visual areas) and higher-level (large-scale neural mechanisms) visual analysis. These differences characterize the distinct visuo-perceptual profile in ASD, broadly described as strengths in detailed, local processing (mediated by lower-level analysis) with or without concurrent difficulty in global processing (mediated by higher-level analysis). This profile has been based largely on isolated levels of processing, providing little information with respect to how performance within the same individual varies with increasing task complexity. It is therefore important to elucidate the relationship between perceptual abilities mediated by different levels of analysis to understand how alterations in the building blocks of perception affect higher-order cognitive and social functions in ASD. We investigated whether enhanced local processing in lower-levels of visual analysis influenced higher-level perception in the same group of children and adolescents with and without ASD by asking the following questions: Does enhanced local processing in low-level perception predict enhanced local performance in high-level perception, specifically for non-social information? Does enhanced local processing in low-level perception influence performance in high-level perception, specifically for social information? A total of 27 and 48 respective children and adolescents with and without ASD performed three tasks, each soliciting a progressively complex visual analysis: (i) low-level perception was assessed by measuring contrast sensitivity to sine-wave, luminance- defined gratings of different spatial frequencies; (ii) high-level perception for non-social information was assessed by measuring local and global reaction times for consistent and inconsistent stimuli in a hierarchical figures task; and (iii) high-level perception for social information was assessed by measuring thresholds in a face-identity discrimination task. Our results revealed that increased sensitivity for high-spatial frequencies in the low-level task predicted faster reaction times in the local condition of the high-level, non-social task. No significant relationship was found between enhanced local performance in the low-level and high-level, social task. The significant association between low- and high-level, non-social tasks suggests that alterations in the "building blocks" of early perception are linked to differences in higher-order visual processes in ASD.

Disclosures: J. Guy: None. **L. Mottron:** None. **C. Berthiaume:** None. **A. Bertone:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.01/F23

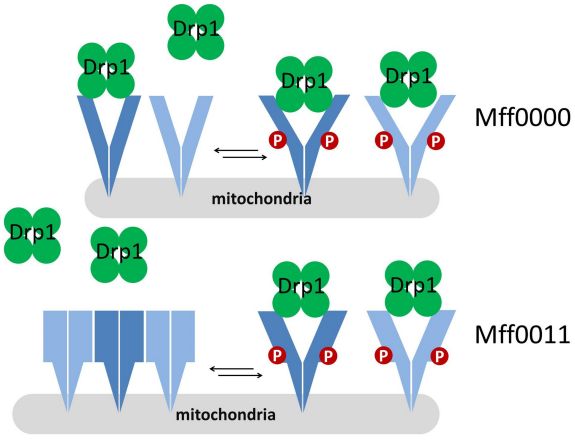
Topic: C.08. Ischemia

Support: NIH R01 NS056244

Title: Regulation of mitochondrial form and function by mitochondrial fission factor (mff) splice variants and phosphorylation

Authors: *R. A. MERRILL[1], T. WILSON[1], N. SRAYOSHI[1], J. WANG[1], Y. KONG[1], J. CRIBBS[1], M. GHONEIM[2], H. N. HIGGS[3], M. SPIES[2], S. STRACK[1,3];
[1]Dept. of Pharmacol., [2]Dept. of Biochem., Univ. of Iowa, Iowa City, IA; [3]Dept. of Biochem., Dartmouth Med. Sch., Hanover, NH

Abstract: Mitochondria are dynamic organelles that undergo tightly regulated fission and fusion processes. Dynamin-related protein 1 (Drp1), a large GTPase, mediates fission by oligomerizing into spirals around the mitochondrion, which contract and physically divide the organelle through GTP hydrolysis. Mitochondrial fission factor (Mff) promotes mitochondrial fission by targeting Drp1 to the outer mitochondrial membrane. Mff has 4 alternatively spliced exons that make up 16 different splice variants having differential tissue expression (0000 lacks all alternative exons, 1111 contains all). The shortest variant, 0000, is the most commonly expressed across different tissues and the most effective at driving fission. Addition of alternative exons leads to less Drp1 recruitment and a decrease in fission. In neurons, the most abundant splice variant is 0011, which promotes less fission than Mff 0000. *In vitro* and in cell data indicate Mff 0000 forms tetramers that diffuse rapidly, while Mff 0011 forms slowly diffusing oligomers of tetramers. Mff is highly phosphorylated at multiple sites and phosphorylation opens the tetramer to enhance Drp1 recruitment and fission. Additionally, phosphorylation of Mff 0011 prevents tetramers from oligomerizing and inhibiting fission. Finally, increased fragmentation resulting from Mff activation leads to an increase to neuronal sensitivity in a culture model of stroke. These results point to Mff as a new target for therapeutic intervention into stroke and neurodegenerative diseases associated with excessive mitochondrial fission.



Disclosures: **R.A. Merrill:** None. **T. Wilson:** None. **N. Srayoshi:** None. **J. Wang:** None. **Y. Kong:** None. **J. Cribbs:** None. **M. Ghoneim:** None. **H.N. Higgs:** None. **M. Spies:** None. **S. Strack:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.02/F24

Topic: C.08. Ischemia

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0002316)

the MRC Program of the MEST/KOSEF (2005-0049477)

Title: Postischemic oral resveratrol attenuates neuronal damage in mice with photothrombotic cerebral ischemia

Authors: *W. LEE, Y. FANG, C. KIM;
Pusan Natl. Univ. Sch. of Med., Yangsan, Gyeongsangnam-Do, Korea, Republic of

Abstract: Resveratrol is a naturally occurring stilbene found in the red grape skin and certain medicinal plants, and is known to provide protection against various infections and stresses. In addition, it has been reported that resveratrol has multiple beneficial health effects such as anticancer, antiviral, anti-inflammatory, anti-ageing and neuroprotective effects. Indeed, most of the protective biological actions associated with resveratrol have been related to its intrinsic radical scavenging properties. In this study, we investigated whether postischemic oral administration of resveratrol reduces photothrombosis-induced cerebral ischemic injury. Male C57BL/6 mice were anesthetized and systemically administered Rose Bengal. Permanent focal ischemia was induced in the medial frontal and somatosensory cortices by irradiating the skull with cold white light. Animals were treated with resveratrol (100 mg/kg, twice) immediately and 20 h after photothrombosis and were sacrificed 24 h after ischemic insult. Resveratrol caused a significant reduction in infarct size and cell apoptosis, and further reduced the expression of IDO, TNF-α and p-STAT2 in the ischemic region. In contrast, resveratrol significantly increased the expression of TrpRS, p-JAK1, p-JAK2 and p-STAT1. These observations suggest that postischemic oral resveratrol can reduce the neuronal damage following photothrombotic cerebral ischemia via modulating the phosphorylation of JAK/STAT families and reducing the inflammatory responses.

Disclosures: **W. Lee:** None. **Y. Fang:** None. **C. Kim:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.03/F25

Topic: C.08. Ischemia

Support: Swiss National Science Foundation

Brain@McGill ZNZ Neuroscience Fund

Title: BDNF "highjacks" the SUMO pathway to induce synaptic GABAergic plasticity after ischemia

Authors: *Z. S. THIROUIN[1,2], R. GILL[3], R. A. MCKINNEY[3], S. K. TYAGARAJAN[1,2]; [1]Inst. of Pharmacol. and Toxicology, Zürich, Switzerland; [2]Neurosci. Ctr. Zurich, Zurich, Switzerland; [3]Dept. of Pharmacol. and Therapeutics, McGill Univ., Montreal, QC, Canada

Abstract: GABAA receptors (GABAARs) mediate most of the fast inhibitory neurotransmission in the CNS, and diverse cellular signaling pathways tightly regulate their availability at the synapse. The recruitment and the clustering of these receptors at the synapse are facilitated by the scaffolding protein gephyrin. Accumulating evidence suggests that gephyrin is subject to diverse post-translational modifications, affecting its scaffolding properties and resulting in changes in GABAergic transmission. We recently identified that gephyrin inter- and intra-molecular interactions that facilitate its synapse scaffolding function are regulated via SUMOylation at specific residues. In the current study, we report that the neurotrophin BDNF facilitates gephyrin SUMOylation under both physiological and pathological conditions to down regulate GABAARs and gephyrin scaffolding at GABAergic synapses. BDNF signaling specifically influences the sub-cellular localization of a subset of SUMO pathway proteins, which in turn modify gephyrin to influence its scaffolding at GABAergic synapses. Furthermore, using an oxygen-glucose deprivation (OGD) paradigm with organotypic hippocampal slice cultures to mimic brain ischemia, we measured functional changes in GABAergic mIPSC amplitude and frequency in CA1 pyramidal neurons. We observed a significant reduction of GABAergic mIPSC frequency but not amplitude 24h post-OGD which returns to baseline after one week. This effect could be effectively prevented using a blocker for BDNF signaling, TrKB-Fc. Interestingly, we could also successfully block BDNF-induced changes at GABAergic synapses by over-expressing a SUMOylation-defective point-mutant of gephyrin. Our data reveal that BDNF signaling modulates the SUMO pathway to induce GABAergic synaptic plasticity in conditions of brain pathology such as ischemia.

Disclosures: Z.S. Thirouin: None. **R. Gill:** None. **R.A. McKinney:** None. **S.K. Tyagarajan:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.04/F26

Topic: C.08. Ischemia

Support: NIH F31 NS073149

NIH RO1 NS081055

The Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Wnt5a regulates differentiation of stroke-responsive neural progenitors

Authors: *A. J. BRUMM, M. MACHNICKI, A. RANDHAWA, J. TOOR, G. COPPOLA, S. T. CARMICHAEL;
Neurol., UCLA, Los Angeles, CA

Abstract: Ischemic stroke induces proliferation of neural progenitor cells (NPC) in the subventricular zone (SVZ) and migration of doublecortin+ neuroblasts to peri-infarct tissue. In a mouse model of distal middle cerebral artery occlusion (dMCAO), stroke-responsive neuroblasts preferentially migrate to angiogenic vasculature within peri-infarct tissue and form a neurovascular niche adjacent to the stroke core. Angiogenesis causally regulates neurogenesis within this niche, but specific angiogenic blood vessel-derived (angiocrine) factors that mediate neuroblast recruitment, survival, and differentiation have not been well described.The secreted protein Wnt5a and multiple putative receptors (Fz2, Ryk) were identified from whole genome expression profiling at 7d after stroke as a novel ligand-receptor(s) system differentially regulated in peri-infarct angiogenic blood vessels and stroke-responsive neuroblasts, respectively. To assess *in vitro* roles for Wnt5a in NPC proliferation and differentiation, neurosphere-expanded NPCs from the adult mouse SVZ were treated with recombinant Wnt5a protein. Wnt5a (10-100 ng/ml for 48 hr) increased proliferation by 27.6% compared to media control, assessed by EdU incorporation from 24-48 hr. When differentiated for 5d, Wnt5a (10 ng/ml) increased MAP2+ neuronal differentiation by 48.2% compared to media control. Wnt5a (10-100 ng/ml) had no significant effect on *in vitro* proliferation or tube formation of mouse brain endothelial cells. To assess an endogenous *in vivo* role for Wnt5a in peri-infarct tissue signaling, lentivirus expressing a Wnt5a-specific miRNA under a ubiquitous EF1α promoter was injected into peri-infarct tissue at the time of stroke. Compared to control virus, Wnt5a knockdown did not affect the number of stroke-responsive neuroblasts in peri-infarct tissue at 14d after MCAO. However, Wnt5a knockdown significantly reduced neuronal differentiation at 60d after stroke, resulting in a 72% decrease in the number of BrdU+NeuN+ mature neurons in peri-infarct tissue (BrdU i.p. d3-10 after MCAO). These results demonstrate that Wnt5a modulates proliferation and differentiation of SVZ-derived NPCs and regulates peri-infarct

neurogenesis after stroke. Ongoing experiments aim to assess the effects of *in vivo* Wnt5a gain of function on NPC proliferation and differentiation and the receptor(s) and downstream signaling involved in SVZ-derived NPC proliferation and differentiation *in vitro*. These studies provide insight into an endogenous signaling system that regulates post-stroke neurogenesis, a process with demonstrated impact on functional recovery from stroke in rodent models.

Disclosures: A.J. Brumm: None. **M. Machnicki:** None. **A. Randhawa:** None. **J. Toor:** None. **G. Coppola:** None. **S.T. Carmichael:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.05/F27

Topic: C.08. Ischemia

Support: NIH NS081299

Title: Aging is associated with suppressed activation of stress response pathways in post-ischemic brains: implications for impaired functional recovery from ischemic stress

Authors: *W. YANG, S. LIU, Z. YU, H. SHENG, W. PASCHEN;
Anesthesiol, Duke Univ. Med. Ctr., Durham, NC

Abstract: Introduction: Aging is associated with increased risk for stroke and impaired functional recovery from transient ischemia. This suggests that the brain's ability to respond to an ischemic challenge declines with age. We, therefore, hypothesized that stress response pathways, enabling cells to withstand stress conditions, become dysfunctional with advanced age. To test our hypothesis and comprehensively analyze the effects of age on the response of brains to a transient interruption of blood supply, we subjected young and aged mice to transient forebrain ischemia, and analyzed a variety of stress response pathways. These included the heat shock response (HSR), the unfolded protein response (UPR), and ubiquitin, SUMO and O-linked β-N-acetylglucosamine (O-GlcNAc) modifications of proteins. Methods: Transient forebrain ischemia was performed on male 2- and 22 -month-old mice. Mice were anesthetized with isoflurane, temperature-controlled, and subjected to 10 minutes common carotid artery occlusion. Changes in mRNA and protein levels were analyzed by qPCR and Western blot, respectively. Statistical analysis was performed by ANOVA followed by Tukey's post-hoc test. Results: Hsp70 expression, Xbp1 splicing, and eIF2α phosphorylation were activated in young and aged mice to the similar extent after ischemia, indicating activation of HSR and UPR. Interestingly, we found that levels of ubiquitin-, SUMO1-, and SUMO2/3-conjugated proteins were markedly increased in brains of young mice, but these responses were significantly suppressed in the cortex and hippocampus of aged mice. Transient ischemia triggered a global

increase in levels of O-GlcNAc modified proteins in young mice, but this stress response was completely absent in brains of aged animals, both in the cortex and hippocampus. Conclusions: We have reported earlier that SUMO2/3 conjugation is dramatically activated after ischemia, and provided evidence that this is a neuroprotective stress response. SUMO proteomics analysis identified SUMOylation-dependent ubiquitin conjugation as a putative neuroprotective pathway that plays a key role in DNA damage repair. Our results suggest that the DNA damage repair pathway may be impaired in old mice. O-GlcNAc modification of proteins has not yet been studied before in models of brain ischemia, but ample evidence supports the notion that activation of O-GlcNAc modification protects heart from ischemic damage. We, therefore, conclude that the inability of aged mice to activate O-GlcNAc modification of proteins in response to transient ischemia could be a critical factor that limits functional recovery from a period of non-sufficient blood supply.

Disclosures: **W. Yang:** None. **S. Liu:** None. **Z. Yu:** None. **H. Sheng:** None. **W. Paschen:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.06/F28

Topic: C.08. Ischemia

Support: Natural Science Foundation of China: 30970664; 31171354

Title: Protective effects of estrogen against progressive lesion of vascular dementia in rats

Authors: ***Y. ZHU**[1], W. ZHANG[1], N. LI[1], Y. DAI[1,2], Q. ZHANG[2], R. WANG[2];
[1]North China Univ. of Sci. and Technol., North China Univ. of Sci. and Technol., Hebei, China;
[2]Med. Col. of Georgia, Department of Neuroscience and Regenerative Medici, GA

Abstract: It is well known that bilateral common carotid artery occlusion (BCCAO) on rat causes chronic hypoperfusion to the brain that is paralleled with a progressive worsening in memory and cognitive function, which ultimately leads to vascular dementia (VD). In current study, we aim to outline the characteristics of middle- and late-stage of vascular dementia and the protective role of long-term treatment with physiological dose of 17β-estrogen (E2). BCCAO was performed on Sprague Dawley (SD) rats at 7 days time interval and E2 was subcutaneously administrated using a mini-pump beginning at unilateral ligation of carotid artery and continued until the end of each experiment. The Morris water maze results showed that the rats underwent BCCAO 3m had a significant impairment in spatial memory ability rather than learning function compared to sham control or E2-treatment animals. The difference could be explained as synaptic- and neuronal ultrastructural-dependent cognitive impairment but not hippocampal

neuron number dependent event, as evidence that 1) BCCAO 3m animals had no statistic difference of the number of NeuN-positive staining both in CA1 and CA3 region compared to sham control or E2-treatment group; 2) the neuron number of Annexin V positive staining, indicating early apoptosis enhanced in BCCAO 3m group compared to sham animals both in CA1 and CA3 region; 3) the ultrastructure damage of neuron including mitochondrial crest broke, demyelination, and decrease of post-synaptic density protein 95 (PSD95) presented in hippocampal CA1 region of BCCAO 3m animals, but not in sham animals; 4) the protein expression of myelin basic protein 2 (MBP2) and PSD 95 significantly attenuated in hippocampal CA1 region of BCCAO 3m group compared to sham control. E2 treatment profoundly reversed the damage induced by BCCAO. However, in 6 m rats after BCCAO, survival neuron significantly decreased and spatial learning and memory deficit compared to sham control animals. And the levels of p-APP, p-tau and Aβ1-42 in BCCAO 6m significantly increased compared to sham animals in both CA1 region and cortex. Continuously administration of E2 markedly prevented the dementia induced by BCCAO. Taken together, the results highlight the characteristics inducing progressive lesions in memory and cognitive function, which BCCAO could cause ultrastrutural-dependent spatial memory storage deficit but not learning ability in early-, or middle-stage of VD, but in 6m after BCCAO, hippocampal CA1 neuronal loss, the accumulation of Aβ1-42 due to vascular dementia. Further study need to be carried out to clarify the molecular mechanisms in the future.

Disclosures: Y. Zhu: None. **W. Zhang:** None. **N. Li:** None. **Y. dai:** None. **Q. zhang:** None. **R. wang:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.07/F29

Topic: C.08. Ischemia

Support: NIH Grant U01 NS057993

NIH Grant U54 NS083932

Title: Ischemic stroke and Neuregulin-1: understanding mechanisms using the Neuroscience Information Framework and biological approaches

Authors: *M. C. SURLES-ZEIGLER[1], A. BANDROWSKI[2], M. MARTONE[2], J. GRETHE[2], Y. LI[1], B. FORD[1];
[1]Neurosci. Inst., Morehouse Sch. of Med., Atlanta, GA; [2]Ctr. for Res. in Biol. Systems, Univ. of California,San Diego, La Jolla, CA

Abstract: Exogenous administration of neuregulin-1 (NRG-1) has been shown to reduce neuronal injury in rat following a middle cerebral artery model of stroke, but the mechanism is not fully understood. Herein we describe our approach to identify biological pathways regulated by NRG-1 in a rodent permanent middle cerebral artery (pMCAO) model of ischemic stroke. We used two approaches to understand these mechanisms: 1) an informatics-based approach where we reanalyzed currently available data about "Neuregulin-1" in about 200 curated biological databases; and 2), an experimental approach using microarray data to test a specific hypothesis. For the informatics approach, we used the Neuroscience Information Framework (NIF), a search engine for neuroscience data, to identify existing data sets and information about NRG-1. NIF is a unique resource that searches hundreds databases allowing users to broadcast a query about a specific topic quickly. A search for the term "NRG1", returned over 63,055 results distributed broadly across over 27 NIF categories. The NIF category, "Pathways" allowed for the identification of 4 pathway databases, BioGRID + interactions, WikiPathways + ChemPathways, CTD + DiseasePathway and KEGG + PathwayGeneOrthologs, returning 458 genes related to NRG1. An enrichment analysis was performed using the Gene Ontology (GO) and the GOrilla database to identify 3 candidate pathways: Fc receptor signaling pathway (43 genes), innate immune pathway (47 genes) and immune response-regulating signaling pathway (45 genes). For the biological approach, rats were allocated to 3 groups (1) NRG-1+ pMCAO (2) Vehicle + pMCAO (3) Sham. Animals were sacrificed 3 hours following surgery, brain tissue of the ipsilateral cortical regions were dissected, RNA isolated and microarray completed with the Affymetrix Rat Genome 2.0st array. Statistical analysis was completed using the R/Bioconductor platform and Cytoscape software (http://www.cytoscape.org/). Our previously published studies also demonstrated that inflammatory pathways were also the most relevant to the effects of NRG-1 24 hours following stroke. However, genes significantly upregulated 3 hours following ischemia and NRG-1 treatment in preliminary studies included Dusp6 (a negative regulator of the MAPK pathway) and protocadherin 6 (involved in the establishment and maintenance of specific neuronal connections in the brain) and associated with the MAPK cascade. We are currently exploring the temporal regulation of gene expression by NRG-1 and its relationship to neuroprotection. Our results demonstrate the potential utility of in silico biology in combination with experimental methods.

Disclosures: M.C. Surles-Zeigler: None. **A. Bandrowski:** None. **M. Martone:** None. **J. Grethe:** None. **Y. Li:** None. **B. Ford:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.08/F30

Topic: C.08. Ischemia

Support: NIH Grant NS081333

Title: Caspase-9 activation in cerebral ischemia does not require MMP-9

Authors: E. CANEPA[1], T. KICHUK[1], A. GEEVARGHESE[1], B. R. CHRISTOPHE[2], E. S. CONNOLLY[2], *C. M. TROY[3];
[1]Dept Pathol, [2]Dept Neurosurg., [3]Dept Pathol & Neurol, Columbia Univ. Medl Ctr., New York, NY

Abstract: Activation of specific caspases - a family of cell death proteases - in cerebral ischemia causes neuronal death. In previous work, we showed that the inhibition of initiator caspase-9 prior to and during stroke reduces neuronal death, infarct volume, and edema, and promotes functional recovery. Ischemic edema is a major cause of mortality during the first 72 hours following stroke, but its mechanisms are poorly understood. Resolving these pathways could unveil therapeutic interventions. To evaluate the role of caspase-9 in the development of edema, we assessed whether the inhibition of caspase-9 in cerebral ischemia reduced edema by measuring blood brain barrier permeability using Evan's blue extravasation and found that inhibition of caspase-9 blocked vasogenic edema. It has been shown by others that matrix metalloproteinase-9 (MMP-9) null animals develop less edema and show less extravasation of Evan's blue during ischemia. It had been proposed that MMPs regulate edema and that activation of MMP-9 occur upstream of caspase activation. Our preliminary studies showed that specific inhibition of caspase-9 abrogated the elevated caspase-9 in the cerebral vasculature and increased MMP-9 expression. To determine the hierarchical relation of caspase-9 and MMP-9 during ischemia we examined the induction of caspase-9 in wild-type and MMP-9 null mice. We find that caspase-9 induction occurs in wild-type and MMP-9 null mice after transient middle cerebral artery occlusion (tMCAo). These data support that caspase-9 acts upstream of MMP-9 in the vasogenic pathway, rather than the converse previously proposed. Ongoing studies are determining how caspase-9 regulates MMP-9 activation in cerebral ischemia. These data suggest a novel mechanism for the induction of vasogenic edema during cerebral ischemia. Thus, targeting caspase-9 in stroke might improve vascular health and provide neuroprotection, which could aid functional recovery.

Disclosures: E. Canepa: None. **T. Kichuk:** None. **A. Geevarghese:** None. **B.R. Christophe:** None. **E.S. Connolly:** None. **C.M. Troy:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.09/F31

Topic: C.08. Ischemia

Support: RO1NS37459

Title: The point mutation uch-l1 c152a protects primary neurons against cyclopentenone prostaglandin-induced cytotoxicity: implications for post-ischemic neuronal injury

Authors: *H. LIU[1,3], M. E. ROSE[3,1], R. W. HICKEY[4], G. UECHI[2], M. BALASUBRAMANI[2], S. H. GRAHAM[3,1];
[1]Neurol., [2]Genomics and Proteomics Core Labs., Univ. of Pittsburgh, Pittsburgh, PA; [3]Geriatric Res. Educational and Clin. Center, VA Pittsburgh Healthcare Syst., Pittsburgh, PA; [4]Pediatrics, Univ. of PIttsburgh Sch. of Med., Pittsburgh, PA

Abstract: Cyclopentenone prostaglandins (CyPGs), such as 15-deoxy-Δ12,14 -prostaglandin J2 (15dPGJ2), are reactive prostaglandin metabolites that exert a variety of biological effects. CyPGs are produced in ischemic brain and disrupt the UPP. Ubiquitin-C-terminal hydrolase L1 (UCH-L1) is a brain-specific deubiquitinating enzyme that has been linked to neurodegenerative diseases. Using MS/MS analysis, we found that the C152 of UCH-L1 is adducted by CyPGs. Mutation of C152 to alanine (C152A) inhibited CyPG modification and conserved recombinant UCH-L1 protein hydrolase activity. Next, a knock-in mouse expressing the UCH-L1 C152A mutation (KI) was constructed with the bacterial artificial chromosome technique. Brain expression and distribution of UCH-L1 in the KI mouse is similar to that of the wild-type mouse. Primary cortical neurons derived from KI mice were resistant to 15dPGJ2 cytotoxicity compared to neurons from wild-type mice as detected by the WST-1 cell viability assay and Caspase-3/ PARP cleavage. This protective effect was accompanied with significantly less ubiquitinated protein accumulation and aggregation in KI primary neurons after 15dPGJ2 treatment. Additionally, 15dPGJ2 -induced axonal injury is also significantly attenuated in KI neurons as compared to wild-type. Furthermore, increased cell viability was observed in post-hypoxia primary neurons from KI mice as compared to wild-type controls with both *in vitro* anoxia and oxygen glucose deprivation models. Taken together, these studies indicate that UCH-L1 function is important in hypoxic neuronal death and the C152 site of UCH-L1 plays a significant role in neuronal survival after hypoxic/ischemic injury.

Disclosures: H. Liu: None. **M.E. Rose:** None. **R.W. Hickey:** None. **G. Uechi:** None. **M. Balasubramani:** None. **S.H. Graham:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.10/F32

Topic: C.08. Ischemia

Title: A novel FBN1 mutation associated with recurrent spontaneous cervical arteries dissections

Authors: *F. CORTINI[1,2], S. LANFRANCONI[3], B. MARINELLI[4], S. CORTI[5], N. BRESOLIN[5], A. BASSOTTI[6];
[1]Genet. Medecine, Ospedale Maggiore Policlinico (milan, Italy), Milano, Italy; [2]Dept. clinical Sci. and community health, [3]IRCCS Fndn. Ca' Granda Ospedale Maggiore Policlinico, Univ. of Milan, Milan, Italy, Italy; [4]Dept. of clinical sciences and community health, Univ. of Milan, Milan, Italy, Italy; [5]Dino Ferrari Centre, Neurosci. Section, Dept. of Pathophysiology and Transplantation (DEPT), Neurol. Unit, IRCCS Fndn. Ca' Granda Ospedale Maggiore Policlinico, Univ. of Milan, Via Francesco Sforza 35, 20122, Milan, Italy., Milan, Italy, Italy; [6]Dept. of clinical Sci. and community heaalth, regional center of Ehlers Danlos syndrome, IRCCS Fndn. Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, Italy

Abstract: Cervical artery dissection (CeAD) represent a major cause of ischemic stroke in young adults. Some rare inherited connective tissue disorders are known to be associated with arterial dissection and may be involved in the pathogenesis of CeAD. We report the case of a 41 years old male who presented sudden onset of vertigo with nausea and vomiting. Neurological evaluation was normal except for rotatory nystagmus. Cerebral CT scan was normal. CT angiography disclosed an acute dissection of the left vertebral artery (V1, V3-V4 segments) and a post-dissection aneurysm of right carotid artery. 24 hours CT evidenced an acute left cerebellar infarction. Anticoagulation was started with full recovery within a few days. Given the presence of multiple spontaneous dissections he underwent molecular analysis in order to disclose a possible underlying collagen pathology. After ruling out COL3A1 and COL4A1 mutations by Sanger sequencing, we extended the molecular analysis taking advantage of a True Seq Custom Panel, developed to improve the diagnostic yield of connective tissue disorders. This custom panel targets the coding regions of 23 genes encoding for collagen isoforms and other extracellular proteins. The enrichment was performed at the Regional Center of Ehlers Danlos Syndrome (Ospedale Maggiore Policlinico, Milan) and sequence runs were generated on a MiSeq (Illumina) platform. We detected a COL6A1 variant c.2669C>T (p.S890L) with uncertain pathogenicity significance and a novel variant c.8008T>C in FBN1 gene, likely resulting in the change of the evolutionary conserved Tyrosine with a Cysteine at codon 2670 (Y2670C). In silico evaluation of the Y2670C mutation, supported its pathogenic value. FBN1 variants in the absence of criteria of Marfan Syndrome have been reported in association with thoracic aortic aneurysm/dissection and bicuspid aortic valve. Our findings enlarge the clinical spectrum of FBN1 mutations and support the importance of genetic screening in selected patients with isolated supra-aortic dissections even in the absence of positive familial history. Connective tissue disorders show genetic and clinical overlap: our study confirms the efficacy of panel sequencing approach to achieve a firm diagnosis even in patients with atypical presentations.

Disclosures: F. Cortini: None. **S. Lanfranconi:** None. **B. Marinelli:** None. **S. Corti:** None. **N. Bresolin:** None. **A. Bassotti:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.11/F33

Topic: C.08. Ischemia

Support: NIH/NINDS research grant R01NS088058

Title: Role of brain-derived estrogen in regulation of pro-survival and pro-apoptotic factors in the hippocampal ca1 region following global cerebral ischemia

Authors: *R. WANG[1], Q. ZHANG[1], R. VADLAMUDI[2], D. BRANN[1];
[1]GEORGIA REGENTS UNIVERSITY, Augusta, GA; [2]Dept. of Obstetrics and Gynecology, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: 17β-Estradiol (E2) is a steroid hormone that has been implicated to exert many beneficial effects in the brain, including neuroprotection, anti-inflammatory effects, and enhancement of synaptic plasticity and cognition. The ovary is well known to be the primary source of E2 generation in the body; however, recent work by our lab and others has shown that aromatase, the enzyme responsible for synthesis of E2 from androgens, is also expressed in the forebrain of the rat, particularly in the hippocampus. To further enhance understanding of local E2 function and roles in the forebrain, we examined aromatase expression in the hippocampus of the ovariectomized rat following 4-vessel global cerebral ischemia (GCI), and determined the effect of knockdown or inhibition of aromatase on neuroprotection, pro-survival and pro-apoptotic factors, and synaptic proteins in the hippocampus. Our results revealed that following GCI, protein expression of aromatase in the hippocampal CA1 region initially decreased at 3h after reperfusion, rebounded at 1day, which was followed by a significant elevation at 3 days and 7 days post-reperfusion, as compared to sham group. Continuous central administration of aromatase antisense oligonucleotide (AS) or aromatase inhibitor letrozole led to enhanced neuronal damage following GCI, as evidenced by an increase in TUNEL-positive cells and a decrease of NeuN-positive cells in the hippocampal CA1 region, as compared to reperfusion 7d and missense control. Furthermore, aromatase-AS treatment abolished the increased phosphorylation level of ERK1/2 and CREB observed at 3h and 1d following GCI. Interestingly, protein levels of the pro-survival factor BDNF were significant attenuated by aromatase-AS treatment, while levels of the pro-apoptotic factors, p-JNK and p-P38, were markedly enhanced by aromatase-AS treatment. Finally, aromatase-AS treatment also significantly reduced expression of the synaptic proteins PSD95 and synaptophysin, indicating a role for local E2 in regulation of synaptic function after GCI. As a whole, the results suggest that local E2 exerts an important pro-survival effect in the hippocampus after cerebral ischemia by regulating the balance of pro-survival and pro-apoptotic factors, as well as modulating expression of key synaptic proteins. (Supported by NIH/NINDS research grant R01NS088058)

Disclosures: **R. Wang:** None. **Q. Zhang:** None. **R. Vadlamudi:** None. **D. Brann:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.12/F34

Topic: C.08. Ischemia

Support: NIH Grant R01 NS082553

Title: Activation of Serpin-1B-mediated apoptotic pathway in focal brain ischemia

Authors: *V. V. DIDENKO;
Neurosurg., Baylor Col. of Med., Houston, TX

Abstract: Serpin 1B-mediated apoptosis is one of the most recently identified apoptotic pathways. Its activation was previously demonstrated in neural tissue development. Here we demonstrate that this apoptotic pathway is also activated in focal brain ischemia. In this presentation we show the co-existence of caspase-dependent and serpin-mediated mechanisms of apoptosis in ischemic brain. The relative contributions of these two different pathways in progression of ischemic injury are discussed. The identification and visualization of serpin1B-mediated apoptosis in tissue sections of experimental stroke in rat model became possible by using the newly-developed *in situ* approach.

Disclosures: V.V. Didenko: None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.13/F35

Topic: C.08. Ischemia

Support: NIH grant R01AG033720

donation by Rose Mary Kubik

Title: Mitochondria/endoplasmic reticulum dysfunction exacerbates oxidative damage in aging white matter ischemia

Authors: *C. BASTIAN, K. STAHON, S. GRIFFITH, G. KIDD, S. BRUNET, S. BALTAN;
Dept. of Neurosci., Cleveland Clin. Fndn., Cleveland, OH

Abstract: White matter (WM) is frequently affected by stroke. Aging WM shows reduced functional recovery following stroke compared to younger WM. Free radicals accumulate with aging due to overproduction or insufficient clearance, resulting in increased oxidative stress. Mitochondria/endoplasmic reticulum (ER) interactions are crucial for protection from oxidative stress. We hypothesized that aging WM is more susceptible to oxidative stress damage mediated by mitochondrial/ER dysfunction, leading to decreased ATP synthesis and impaired Ca^{2+} homeostasis. Mouse optic nerves (MON) obtained from C57BL/6J and Thy-1 CFP(+) mice at 1 month (young) or 12 months (aging) of age were used. Axon function was quantified by recording evoked compound action potentials. Mitochondrial/ER structure and interactions were determined by 3D-electron microscopy, Western blotting, ATP assays, and CFP (+) fluorescent mitochondrial imaging. Oxidative stress was assessed by nitric oxide synthase (NOS) and glutathione assays and by Western blotting for oxidative stress markers (3-NT and 4-HNE). Oxygen-glucose deprivation (OGD) for 60 min resulted in decreased functional recovery of aging WM compared to young WM. Aging WM displayed elevated NOS activity and increased levels of by-products of lipid peroxidation (4-HNE) and protein nitration (3-NT), indicating aggravated reactive oxygen and nitrogen species mediated damage. Structurally, compared to young axons, aging axons were larger, with thicker myelin, and were characterized by longer and thicker mitochondria due to altered levels of mitochondrial shaping proteins. This was further confirmed by 3D-EM and CFP (+) imaging and may compensate for the decreased ATP levels detected in aging WM. Moreover, mitochondrial-ER interactions were compromised due to decreased association between the organelles and due to decrease in levels of mitochondrial trafficking protein miro-2, which suggests defective Ca^{2+} homeostasis in aging axons. WM exposed to OGD showed ultrastructural changes such as axonal and mitochondrial swelling, loss of cristae, and a decrease in ATP levels. Calnexin, an ER stress response chaperone protein, was decreased under baseline conditions in aging axons, but did not show upregulation following OGD when compared to young axons, suggesting a defect in unfolded protein responses. We conclude that aging WM is increasingly vulnerable to stroke because of inherent structural changes in axons and mitochondria/ER interactions, leading to depletion of ATP and defective Ca^{2+} dynamics, resulting in increased oxidative stress.

Disclosures: C. Bastian: None. **K. Stahon:** None. **S. Griffith:** None. **G. Kidd:** None. **S. Brunet:** None. **S. Baltan:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.14/F36

Topic: C.08. Ischemia

Support: NIH grant HL081752

NIH grant HL66296

NIH grant R00AT004197

Title: Transgenic mice overexpressing human angiotensin i receptor gene are susceptible to stroke injury

Authors: *J. TULSULKAR[1], S. JAIN[2], A. RANA[2], A. KUMAR[2], Z. SHAH[1];
[1]Medicinal biochemistry, [2]Physiol. and phamacology, Univ. of Toledo, Toledo, OH

Abstract: Hypertension is one of the co-morbid conditions for stroke and profoundly increases its incidence. Angiotensin II (AngII) is shown to be at the center stage in driving the renin angiotensin system via activation of angiotensin 1 receptor (AT1R). This makes the AT1R gene one of the candidates whose differential regulation leads to the predisposition to disorders associated with hypertension. A haplotype block of four SNPs is represented primarily by haplotype-I, or Hap-I (TTAA), and haplotype-II, or Hap-II (AGCG), in the promoter of human AT1R (hAT1R) gene. To better understand the physiological role of these haplotypes, transgenic (TG) mice containing Hap-I and Hap-II of the hAT1R gene in a 166-kb bacterial artificial chromosome (BAC) were generated. Mice received injection of endothelin-1 (1 mg/ml) directly in to the striatum and were evaluated for neurologic deficit scores and sacrificed for analysis of infarct volume and mRNA levels of various proteins. Mice containing Hap-I suffered from significantly higher neurological deficits and larger brain infarcts than Hap II. Similarly, the molecular analysis of oxidant and inflammatory markers in brains of mice showed a significant increase ($p < 0.05$) in NOX-1 (2.3-fold), CRP (4.3-fold), and IL6 (1.9-fold) and a corresponding reduced expression of antioxidants SOD (60 %) and HO1 (55 %) in Hap-I mice as compared to Hap-II mice. These results suggest that increased expression of hAT1R rendered Hap-I TG mice susceptible to stroke-related pathology, possibly due to increased level of brain inflammatory and oxidative stress markers and a suppressed antioxidant defense system.

Disclosures: **J. Tulsulkar:** None. **S. Jain:** None. **A. Rana:** None. **A. Kumar:** None. **Z. Shah:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.15/F37

Topic: C.08. Ischemia

Support: The Scientific and Technological Research Council of Turkey (TÜBİTAK) project no:114S190

7th Framework Programme EU Marie Curie Actions

Co-funded Brain Circulation Scheme; The Scientific and Technological Research Council of Turkey (TÜBİTAK

Title: Ischemia/reperfusion injury leads to upregulation of contractile proteins of pericytes

Authors: *S. YILMAZ OZCAN[1], L. ALARCON-MARTINEZ[1], B. DONMEZ-DEMIR[1], T. DALKARA[1,2], M. YEMISCI[1,2];
[1]Inst. of Neurolog. Sci. and Psychiatry, Hacettepe, Ankara, Turkey; [2]Fac. of Medicine, Dept. of Neurology, Hacettepe Univ., Ankara, Turkey

Abstract: Pericytes are located on the walls of the pre-capillary arterioles, post-arteriole capillaries and post-capillary venules, wrapping them with their processes. They have many functions in health and disease such as maintenance of the blood-brain barrier, regulation of microcirculation, phagocytosis, and angiogenesis. True capillary (<4μm) pericytes are shown to have a major role in regulation of microcirculatory blood flow in the brain and retina. The contractile abilities are currently disputed. With a novel histological and immunohistochemical method, we showed that alpha-smooth muscle actin (alpha-SMA) which plays a central role in the contractile response of large arteries and arterioles, can be detected in pericytes even in the smallest capillaries of retina and brain of adult mice. We also found that in retinal ischemia done by central retinal artery occlusion or transient Middle Cerebral Artery occlusion (MCAo) made by intraluminal filament method, alpha-SMA mediated capillary constrictions emerge. We also demonstrated by quantitative RT-PCR that alpha-SMA mRNA increased dramatically in ischemic territory after 2 hours ischemia and 6 hours reperfusion of MCAo mice compared to sham operated and naive animals. In addition to alpha-SMA expression, when we analyzed the expression of other contractile genes such as desmin and vimentin, we observed a significant up-regulation in the ischemic MCA territory. The increases in mRNA expressions of these three genes were not statistically significant after 2 hours of ischemia and 3 hours of reperfusion condition. In conclusion, we detected alpha-SMA expression in pericytes, which is induced after ischemia consistent with up-regulation of various contractile genes related to contraction. The question of whether this early increase in pericyte contraction markers is an indication of early angiogenesis or other pathophysiological role played by pericytes in the setting of cerebral ischemia is the subject of our further investigations.

Disclosures: S. Yilmaz Ozcan: None. **L. Alarcon-Martinez:** None. **B. Donmez-Demir:** None. **T. Dalkara:** None. **M. Yemisci:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.16/F38

Topic: C.08. Ischemia

Support: NIH Grant NS34179

AHA Grant 10SDG2600298

Title: Mass spectrometry analysis of post-ischemic insoluble proteins reveals preferential accumulation of proteins involved in RNA/protein synthesis and cell signaling

Authors: *K. HOCHRAINER[1], A. KAHL[1], K. JACKMAN[1], J. BASKAR[1], S. ZHANG[2], J. ANRATHER[1], C. IADECOLA[1];
[1]Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [2]Inst. of Biotech. and Life Sci. Biotechnologies, Cornell Univ., Ithaca, NY

Abstract: Brain injury is frequently associated with accumulation of insoluble protein inclusions (Ross and Poirier, 2004). Although the protein composition of the inclusions is unique for each injury modality, ubiquitin is frequently present (Dantuma and Bott, 2014). We showed that focal cerebral ischemia results in the formation of ubiquitin-positive protein inclusions (Hochrainer et al, 2012). Unlike neurodegeneration, in which irreversible ubiquitin-containing inclusions are formed over a long time and may portend cell death, after ischemia ubiquitination is rapid, transient and does not predict brain damage (Hochrainer et al, 2012). To gain insight into the biological significance of protein inclusions after ischemia we sought to identify the contained proteins. Mice underwent middle cerebral artery occlusion or sham-surgery (n=8/ group). After reperfusion for 1 hour, when ubiquitin is maximally detected in inclusions, mice were sacrificed and insoluble proteins, as determined by their resistance to 2% Triton X-100 solubilization, were obtained from ischemic neocortices. Isolated proteins were digested with trypsin and identified by nanoLC-MS/MS (n=4, each 2 pooled sham and ischemia animals). Relative amounts of proteins detected in sham and ischemia groups were quantified for each run using label-free quantification (MaxQuant). Ischemia versus sham fold change was determined and mean values were calculated across all 4 runs. Proteins with fold change values within the internal standard range (trypsin: 1.04±0.16) were considered unchanged (131, fold change 1.20 - 0.88); 197 proteins were increased (fold change 1.21 - 6.24x10e7) and 213 were decreased (fold change 0.87 - 8.46x10e-8) in the insoluble fraction after ischemia/reperfusion. As anticipated, ubiquitin was among the most increased proteins (5.83x10e7±2.91x10e7). To place the groups of proteins into a biological context we analyzed gene ontology term enrichment using the DAVID (http://david.abcc.ncifcrf.gov) database. Proteins that were unchanged or reduced by the ischemic challenge were mainly clustered around membrane, structural molecule, organelle and macromolecular complex terms ($P<0.001$). However, proteins that were increased associated with DNA/RNA binding, RNA processing and signal transduction ($P<0.001$). Such distinct enrichment of proteins involved in RNA/protein synthesis and cell signaling in post-ischemic ubiquitin-rich inclusions is consistent with a protective mechanism by which proteins sub-serving vital cellular functions are sequestered in inclusions to shield them from the detrimental cellular environment associated with cerebral ischemia.

Disclosures: **K. Hochrainer:** None. **A. Kahl:** None. **K. Jackman:** None. **J. Baskar:** None. **S. Zhang:** None. **J. Anrather:** None. **C. Iadecola:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.17/F39

Topic: C.08. Ischemia

Support: MINECO (Reference BIO2013-49006-C2-2-R)

Universidad de Leon fellowship for Enrique Font.

Title: Salubrinal post-ischemic treatment effect on the tumor necrosis factor receptor 1 (TNFR-1) pathways

Authors: *E. FONT BELMONTE[1], B. ANUNCIBAY-SOTO[1], M. SANTOS-GALDIANO[1], I. F. UGIDOS[1], M. REGUEIRO-PURRIÑOS[3], J. M. GONZALO-ORDEN[2], A. FERNÁNDEZ-LÓPEZ[1];
[1]Area de Biologia Celular., [2]Area de Medicina y Cirugia Animal, Univ. De Leon. Inst. De Biomedicina, Leon., Spain; [3]Area de Medicina y Cirugia Animal, Facultad de Veterinaria, Leon, Spain

Abstract: Here we report the effect of post-ischemic salubrinal treatment on the tumor necrosis factor receptor 1 (TNFR-1) pathways using a model of global cerebral ischemia. A two vessel occlusion for 15 minutes, at 45-50 mmHg blood pressure maintained by exanguination, followed by 24 h reperfusion was performed. One hour after the insult, a single dose (1 mg/kg) was injected intraperitoneally. The effects of salubrinal on the different possible pathways (apoptosis, necroptosis or cell survival) ignited by TNFR1 activation were analyzed by qPCR, Western blot or immunostaining in hippocampal CA1 and CA3 as well as the cerebral cortex. TNFR1, caspase 8, cIAP1, IκB, A20, MLKL, RIP1, RIP3, cFLIP, CYLD, NFκB were measured as parameters of these pathways and the most striking effect of the salubrinal treatment was a decrease in the expression of TNFR-1 transcripts 24 h after insult. Our data suggest that salubrinal treatment enhances cell survival and decreases the cell death pathways elicited by TNFR1. This study was supported by MINECO (reference BIO2013-49006-C2-2-R) who also supports Berta Anuncibay-Soto and María Santos-Galdiano. Universidad de Leon fellowship for Enrique Font.

Disclosures: E. Font Belmonte: None. **B. Anuncibay-Soto:** None. **M. Santos-Galdiano:** None. **I.F. Ugidos:** None. **M. Regueiro-Purriños:** None. **J.M. Gonzalo-Orden:** None. **A. Fernández-López:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.18/F40

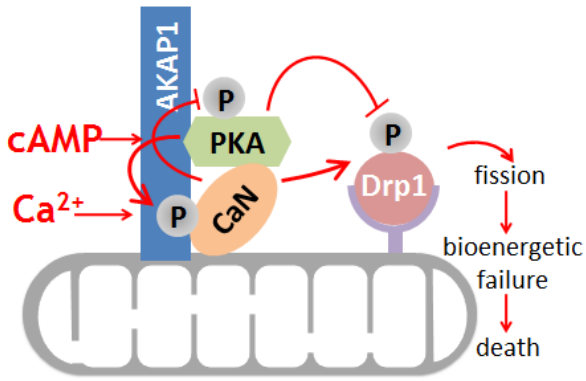
Topic: C.08. Ischemia

Support: R01 NS056244

Title: The role of interplay between AKAP1 complex with CaN and PKA in mitochondrial morphology and neuronal survival

Authors: *Y. LIU[1], R. A. MERRILL[2], A. SLUPE[2], K. FLIPPO[2], A. CHAUHAN[3], S. MCKNIGHT[4], S. STRACK[2];
[1]Univ. of Iowa Carver Col. of Med. Depa, Iowa City, IA; [2]Dept. of Pharmacol., [3]Oncology, and Blood & Marrow Transplantation, Univ. of Iowa Carver Col. of Med., Iowa City, IA; [4]Dept. of Pharmacol., Univ. of Washington Sch. of Med., Seattle, WA

Abstract: Mitochondria play important roles in both early (minutes) and late (days) stages of ischemic brain injury and offer attractive targets for development of stroke therapy. Mitochondrial architecture, a product of opposing fission and fusion reactions, shows signs of extreme fragmentation during stroke. Mitochondrial fission catalyzed by the mechanoenzyme dynamin-related protein 1 (Drp1) is tightly regulated through phosphorylation of a highly conserved Drp1 phosphorylation site (S637). When this site is phosphorylated by protein kinase A (PKA), mitochondria fusion is unopposed. PKA is antagonized by the Ca2+-dependent protein phosphatase calcineurin (CaN or PP2B), which dephosphorylates Drp1 at S637 to promote mitochondrial fragmentation and dephosphorylated Drp1 sensitizes PC12 cells to apoptosis.Mitochondria-localized A kinase anchoring protein 1 (AKAP1, aka D-AKAP1, AKAP121, AKAP149) displays potent neuroprotective properties. AKAP1 assembles a mitochondrial “signalosome” by binding both PKA and CaN that integrates death and survival signals through reversible phosphorylation of Drp1 at S637 to restructure the organelle. Using AKAP1 pulldowns, we determined CaN binds to the N-terminus of AKAP1 and this interaction was stabilized via the CaN interaction with a PKA regulator subunit bound to AKAP1. Furthermore, phosphorylation of AKAP1 by PKA may increase the CaN: AKAP1 interaction. By expressing AKAP1 mutants in primary hippocampal neurons, we demonstrate that CaN binding-deficient AKAP1 mutant result in mitochondria elongation while PKA binding-deficient AKAP1 mutant resulted in mitochondrial fragmentation. These findings also correspond to the neuron susceptibility to excitotoxic insult. Finally, mice lacking AKAP1 had smaller mitochondria, age-dependent neurodegeneration and a pronounced increase in infarct volumes following middle cerebral artery occlusion. These data suggest that AKAP1 play a critical role in regulating mitochondrial morphology and neurons are more susceptible to excitotoxic insults without proper AKAP1 activities.



Disclosures: **Y. Liu:** None. **R.A. Merrill:** None. **A. Slupe:** None. **K. Flippo:** None. **A. Chauhan:** None. **S. McKnight:** None. **S. Strack:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.19/F41

Topic: C.08. Ischemia

Title: Galectin-3: madolator of microglia function

Authors: ***R. RAHIMIAN**[1], E. ABDELHAMID[2], S. SATO[3], L. SCHLICHTER[4], S. LIVELY[4], J. KRIZ[5];
[1]Inst. Universitaire en Santé Mentale de Québec, Laval Univ., Quebec, QC, Canada; [2]Universitaire en Santé Mentale de Québec, and Dept. of Psychiatry and Neurosci., Quebec, QC, Canada; [3]Lab. of Glycobiology and Bioimaging, Res. Ctr. for Infectious Diseases, Dept. of Microbiology and Immunology, Fac. of Medicine, Laval Univ., Quebec, QC, Canada; [4]Genes and Develop. Division, Toronto Western Res. Institute, Univ. Hlth. Network Toronto, ON, Canada ; Dept. of Physiology, Univ. of Toronto Toronto, Toronto, ON, Canada; [5]Inst. Universitaire en Santé Mentale de Québec, and Dept. of Psychiatry and Neurosci., Quebec, QC, Canada

Abstract: Background: Galectin-3 (Gal-3), a member of β-galactosides-binding lectins, has recently emerged as a molecule with immunoregulatory functions. Gal-3 is a pleiotropic protein involved in cell adhesion, activation, proliferation, apoptosis and cell migration. When liberated by macrophages including activated microglia, it exerts strong chemotactic properties on monocytes and macrophages and contributes to phagocytosis in neutrophils and macrophages. Of note, recent investigations have revealed that Gal-3 is required for resident microglia activation and proliferation in response to ischemic insult. So far research addressing a regulatory role for

Gal-3 in microglia morphology is lacking. In this study, We investigated the effects of Gal-3 on microglia function. Given the preferential expression of Gal-3 in glia and its prominent role in inflammatory/remodeling events, Gal-3 might serve as a potential target in neuroinflammatory and neurodegenerative settings. **Methods:** Primary microglia cell cultures, challenged with Gal-3, were exploited for the assessment of morphology and secretory profile using real-time PCR and immunofluorescence while migration was studied using scratch-wound assay. **Results:** *In vitro* measurements revealed that 24 hour-exposure of primary microglia with Gal-3 increased the number and length of Filopodia via. Moreover, at transcriptional level, Gal-3 down-regulated pro-inflammatory cytokines (TNF-a and IL-1b) and upregulated anti-inflammatory cytokine (IL-4) and IGF-1. Interestingly, Gal-3 augmented resting microglia migration 1 day after treatment. **Conclusions:** Our results, *in vitro* and *in vivo*, demonstrate that Gal-3 induces microglial ramification. Gal-3 also modulates the cytokine profile of microglia toward an anti-inflammatory state. Together, these findings suggest the role of Gal-3 as a fine-tuner of microglia cytoskeleton and secretory profile. Our onging investigations in our lab interrogate signaling pathways involved.

Disclosures: R. Rahimian: None. **E. Abdelhamid:** None. **S. Sato:** None. **L. schlichter:** None. **S. Lively:** None. **J. Kriz:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.20/F42

Topic: C.08. Ischemia

Support: AHA 10GRNT4710004

Title: Deletion of TRPC6 attenuates nmda receptor-mediated Ca^{2+} entry and Ca^{2+}-induced neurotoxicity in models of cerebral ischemia

Authors: J. CHEN, Z. LI, S. CHAN, *Z. CHENG;
Burnett Sch. of Biomed. Sci., Univ. of Central Florida, Orlando, FL

Abstract: Ischemic stroke is a major cause of death and disability in adults. TRPC6 is a non-selective cation channel which is permeable to Na+ and Ca2+. Recent data indicated that the TRPC6 expression was increased in stroke and that the amount of ischemia/reperfusion (I/R) induced brain damage measured 3 days after I/R was significantly less in TRPC6-/- mice than in WT mice. This seemed to indicate that TRPC6 gene disruption rendered the brain less susceptible to ischemic insults. However, the molecular mechanism by which TRPC6 deletion protected against ischemic brain injury was unknown. According to the literature, the AMPA-NMDA pathway is considered as a major subset of the multiple routes of ionic imbalance that

are induced in brain injury and neurodegeneration. Thus, we hypothesized that TRPC6 deletion prevents Ca2+ overload by altering NMDAR channel activity, which may protect against oxygen-glucose-deprivation (OGD)-induced cell death in primary cultured cortical neurons. In this study, we have demonstrated that deletion of TRPC6 reduces oxygen-glucose deprivation (OGD) and glutamate/NMDA excitoxicity-induced cortical neuron death. In addition, we have shown that OGD increased, but deletion of TRPC6 decreased NMDAR-dependent Ca2+ influx, which is regulated by Na+ entry via both voltage sensitive and non-voltage sensitive Na+ channels. Along with the previous work, we may conclude that cerebral ischemia-induced brain damage is, in part, due to upregulation of TRPC6 ion channels in cortical neurons. Overexpression of TRPC 6 during ischemia and OGD may induce cortical neuronal death through possible TRPC6 dependent Na+ entry which activates NMDA receptors, thus leading to overload of Ca2+. OGD- and glutamate/NMDA excitotoxicity-induced cell death *in vitro* are likely mediated through both TRPC6-NMDA and AMPA-NMDA pathways, and deletion of TRPC6 protects cerebral ischemia-induced brain damage *in vivo*. These findings may provide a potential target for future interventions in stroke-induced brain damage.

Disclosures: J. Chen: None. **Z. Li:** None. **S. Chan:** None. **Z. Cheng:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.21/F43

Topic: C.08. Ischemia

Support: Heart and Stroke Foundation of Canada (HSFC)

Canadian Partnership for Stroke Recovery (J.-C.-B., H.-H.C., D.C.L.)

Heart and Stroke Foundation of Ontario Mid-Career Investigator Award (H.-H.C.)

Title: IRF2BP2 necessity in innate immune response affecting stroke recovery in mice

Authors: *S. A. CRUZ[1,2], A. HARI[1,2], Z. QIN[2], X. ZHOU[2], C. CHANG[2], J. BUI[2], A. F. R. STEWART[3,4,5], H.-H. CHEN[2,1,5];
[1]Dept. of Cell. and Mol. Med., Ottawa, ON, Canada; [2]Ottawa Hosp. Res. Inst., Ottawa, ON, Canada; [3]Dept. of Biochemistry, Microbiology and Immunol., Ottawa, ON, Canada; [4]Univ. of Ottawa Heart Inst., Ottawa, ON, Canada; [5]Dept. of Medicine, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Immune response and inflammatory signaling are intertwined events after stroke, the second most frequent cause of death and debilitating illness. Microglia, the resident immune cell in brain can be polarized into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes

after brain injury. Initial M1 inflammatory signaling subsides by turning on M2 reparative actions but nonresolving inflammation causes further tissue damage. Members of the IRF family of transcription factors play a major role for innate and adaptive immune responses regulating genes encoding IFNα, IFNβ and IFNγ, including IFNs-inducible genes. Supporting evidence showed IRF2 activates M1 polarization, while IRF2 gene expression and transactivational activities was known to be inhibited by IRF2 binding protein 2 (IRF2BP2). In this study, we hypothesize that IRF2BP2 serves as modulator/suppressor of IRF2 action on microglia/macrophages polarization. Using an animal model lacking IRF2BP2 in myeloid cell-lineage that includes peripheral macrophages and CNS microglia, we are testing whether IRF2BP2 action is neuroprotective or –destructive after CNS focal ischemia. In adult mice, the infarct volume of IRF2BP2$^{-/-}$ mice 4 days after permanent focal ischemia by cerebrocortical photothrombosis was significantly larger compared to IRF2BP2$^{+/+}$ mice. This was supported by behavioral analysis using cylinder and adhesive removal tests, showing IRF2BP2$^{-/-}$ mice have significant deficit of the contralateral forelimb usage compared to IRF2BP2$^{+/+}$ mice. At the same time, the number of cells labeled by anti-Iba1, a marker protein of microglia and macrophages was found to be significantly lower in IRF2BP2$^{-/-}$ compared to IRF2BP2$^{+/+}$, indicating a compromised immune cell build-up after brain ischemia. *In vitro*, cell culture of bone marrow derived macrophages (BMDM) from IRF2BP2$^{-/-}$ mice showed attenuated gene expression of cytokines that marked M1 and M2 polarization compared to IRF2BP2$^{+/+}$ mice. Furthermore, fluorescence activated cell sorting (FACS) analysis after 4d stroke showed lower M1 phenotype in IRF2BP2$^{-/-}$ mice. While the microglia phenotypes can be used as an indicator of the signaling involved during an immune response, our data apparently shows that the loss of IRF2BP2 can result into increased brain cell death and affects microglia population at the site of damage. In conclusion, our study provides novel evidence of a critical role for IRF2BP2 for recovery from the detrimental aftermath of stroke. Further study of IRF2BP2 mechanisms of action in stroke may provide beneficial therapeutic ways of dealing with recovery period.

Disclosures: **S.A. Cruz:** None. **A. Hari:** None. **Z. Qin:** None. **X. Zhou:** None. **C. Chang:** None. **J. Bui:** None. **A.F.R. Stewart:** None. **H. Chen:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.22/F44

Topic: C.08. Ischemia

Support: Edwin W. and Catherine M. Davis Foundation

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Molecular mechanisms of axonal sprouting in a cortical circuit induced by limb overuse after stroke

Authors: *E. H. NIE[1], G. COPPOLA[2], S. T. CARMICHAEL[3];
[1]Neurol., UCLA, Los Angeles, CA; [2]Psychiatry and Biobehavioral Sci., [3]Neurol., UCLA David Geffen Sch. of Med., Los Angeles, CA

Abstract: Stroke survivors worldwide are left with permanent sensorimotor and cognitive disabilities for which there are no medical treatments. However, a behavioral paradigm of limb overuse after stroke, known as constraint-induced movement therapy (CIMT), has been shown in clinical trials to result in significant motor improvements. The goal of the current project is to understand how limb overuse shapes cortical circuit reorganization after ischemic stroke, and how specific gene systems drive this important neural repair process. These studies utilize a mouse model of stroke and forelimb overuse that approximates human CIMT. In this model, fluorescent neuronal tracers were used to quantitatively map cortical neurons that connect to premotor cortex upon post-stroke limb overuse. We find that recovery involves the activity-dependent formation of connections between premotor cortex (PMC) and retrosplenial cortex (RSC), a parietal area involved in spatial learning. The finding was significant and replicated across two independent cohorts ($p<0.05$, Hotelling's T test). Moreover, retrograde corticospinal tract tracing confirms that the activity-induced connections are anatomically distinct from layer V corticospinal motor areas. Next, tracer-labeled cells in RSC and matched control neurons were FACS purified for RNA-Seq. Pathway analyses indicate that limb overuse induces canonical signaling in calcium signaling, cell cycle, and Ephrin A pathways. A top network hit was identified in cell growth, proliferation and tissue development. The RSC-PMC circuit is characterized by circuit-specific expression of activity-induced and growth-related genes. From the relatively small group of 162 genes that are regulated by limb overuse, top candidates have been statistically prioritized (FDR<0.1, $p<0.005$) for in-vitro neuronal outgrowth screening using primary cortical neurons. Current knockout experiments in-vitro have shown that gRNAs directed against these genes can facilitate CRISPR/cas9 gene-editing of these candidates. Cel-I mutation detection has confirmed gene knockout for axonal outgrowth studies. Ongoing design of an AAV-mediated cas9 for in-vivo delivery aims to target the prioritized candidates that increase neuronal outgrowth from the in-vitro screening dataset. Quantitative cortical mapping will examine how the genetic modifications affect the RSC-PMC circuit mapped in the first phase of this study. Through single and multiplexed genetic gain and loss-of-function studies, we now hope to understand how injury and activity-dependent molecular pathways converge in the RSC-PMC circuit during limb overuse and brain repair after stroke.

Disclosures: E.H. Nie: None. **G. Coppola:** None. **S.T. Carmichael:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.23/G1

Topic: C.08. Ischemia

Support: NIH grant R01AG033720

donation by Rose Mary Kubik

Title: Aging upregulates Ca^{2+}-permeable AMPA receptors and renders them the predominant mediators of injury during white matter stroke

Authors: *S. BALTAN, S. GRIFFITH, C. BASTIAN, B. SCOTT, S. BRUNET;
Dept. of Neurosciences, Cleveland Clin., Cleveland, OH

Abstract: Stroke risk increases with age. The human brain is comprised of equal portions of gray matter and white matter (WM) and both are injured during stroke. AMPA/kainate glutamate receptors mediate excitotoxic injury and it was shown that blockade of AMPA and kainate receptors with a broad spectrum glutamate receptor blocker, NBQX (30 μM), preserves axon function and promotes recovery following ischemia in young and aging WM. Using selective glutamate receptor blockers, we investigated whether excitotoxic injury is mediated via age-specific glutamate receptor subtypes. Mouse optic nerves (MONs) obtained from male C57BL/6J mice at 1 month or 12 months of age were used to determine the cellular expression and labeling intensity of the Ca^{2+}-permeable AMPA receptor subunit GluR4. GluR4 subunit immunoreactivity was observed on GFAP (+) astrocytes and APC (+) oligodendrocytes increased with aging, suggesting that Ca^{2+}-permeable AMPA receptors may become predominant in mediating ischemic injury in aging WM. Axon function was quantified while exposing MONs to oxygen-glucose deprivation (OGD) for 60 minutes. Young MONs typically recovered better after OGD than aging MONs. Blocking Ca^{2+}-permeable AMPA receptors (NASPM, 10μM) or kainate receptors (UBP 302, 1 μM) individually provided no protection in young WM (OGD: 20.0 ± 2.1%, n=14; AMPA: 23 ± 2.0%, n = 5; kainate: 22 ± 4.4%, n = 9). In contrast, blockade of Ca^{2+}-permeable AMPA receptors resulted in significant recovery in aging WM, while blockade of kainate receptors showed more modest recovery (OGD: 10.9 ± 1.1%, n=9; AMPA: 38.8 ± 6.4%, n= 3, $p<0.001$; kainate: 24 ± 4.3%, n=5, $p<0.01$). On the other hand, blocking both the Ca^{2+}-permeable and Ca^{2+}-impermeable AMPA receptors with GYKI (30μM) preserved axon function in young as well as aging WM (Young: GYKI, 34.5 ± 3.3, n=5, $p<0.01$; Aging: 37.7 ± 3.8, n=7, $p<0.001$). Combined blockade of Ca^{2+}-permeable AMPA and kainate receptors preserved both young and aging WM (Young: 50 ± 7.8%, n=4, $p<0.05$; Aging: 40.5 ± 9.4%, n=6, $p<0.001$). Finally, broad spectrum glutamate receptor blockade with NBQX showed axon recovery similar to blocking with both NASPM and UBP302 in young and aging WM (Young: 51± 3.5, n=8, $p<0.001$; Aging: 40.8 ± 1.6, n=10, $p<0.001$). Our results suggest that in young WM, activation of either AMPA or kainate receptors mediate ischemic injury, while in aging WM, activation of Ca^{2+}-permeable AMPA receptors are primarily responsible for ischemic WM injury.

Disclosures: **S. Baltan:** None. **S. Griffith:** None. **C. Bastian:** None. **B. Scott:** None. **S. Brunet:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.24/G2

Topic: C.08. Ischemia

Support: NIH Grant GM044842

Title: Differential activity of aminopeptidase N (EC 3.4.11.2) contributes to selective vulnerability of Cornu Ammonis 1 to oxygen-glucose deprivation in the rat hippocampus

Authors: ***Y. OU**, S. WEBER;
Chem., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The selective vulnerability of the hippocampus proper to oxygen-glucose deprivation (OGD) has been known for over a century, with the Cornu Ammonis 1 (CA1) region being more susceptible to neuronal cell death than the CA3 (Stanika et al., 2010). Ectopeptidases are membrane-bound enzymes whose catalytic domains face the extracellular space (ECS). They are conventionally viewed as having a clearance role, but recently they have been shown to modulate peptide levels in response to either pathological (e.g. stress) or trophic triggers (e.g. preconditioning) (reviewed in Ou et al., 2014). We developed a novel sampling tool called electroosmotic push-pull perfusion (EOPPP) that introduces exogenous neuropeptide substrates into the ECS and collects hydrolysis products with good spatial resolution (Rupert et al., 2013). This method was used to measure ectopeptidase activity in the CA1 and CA3 of rat organotypic hippocampal slice cultures (OHSCs). We demonstrated that higher activity of bestatin-sensitive Leu-enkephalin-hydrolyzing aminopeptidase N contributes to CA1 vulnerability to OGD. Inhibition of this enzyme significantly reduced CA1 damage relative to that in CA3 as a result of OGD. *References:* Ou Y, Wu J, Sandberg M, Weber S (2014) Electroosmotic perfusion of tissue: sampling the extracellular space and quantitative assessment of membrane-bound enzyme activity in organotypic hippocampal slice cultures. Anal Bioanal Chem 406:6455-6468. Rupert AE, Ou Y, Sandberg M, Weber SG (2013) Electroosmotic Push–Pull Perfusion: Description and Application to Qualitative Analysis of the Hydrolysis of Exogenous Galanin in Organotypic Hippocampal Slice Cultures. ACS Chemical Neuroscience 4:838-848. Stanika RI, Winters CA, Pivovarova NB, Andrews SB (2010) Differential NMDA receptor-dependent calcium loading and mitochondrial dysfunction in CA1 vs. CA3 hippocampal neurons. Neurobiology of disease 37:403-411.

Disclosures: **Y. Ou:** None. **S. Weber:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.25/G3

Topic: C.08. Ischemia

Support: NIH Grant R01NS073832 (AZ)

NIH Grant R01NS066027 (ZGX)

NIH Grant U54NS08932 (MSM Neuroscience)

Title: Neuroprotection against ischemic cell death - a novel role of polycomb group protein EZH2

Authors: P. SHARMA, T. LENG, Z.-G. XIONG, Z. YANG, R. SIMON, *A. ZHOU; Morehouse Sch. of Med., Atlanta, GA

Abstract: Polycomb group (PcG) proteins are transcriptional repressors, regulating a broad spectrum of genes through epigenetic mechanisms. PcG proteins are assembled into multi-protein Polycomb Repressive Complexes (PRC) and exert their function by concerted modifications of histone proteins. Previous studies have shown critical roles of several PcG proteins of the PRC1 complex in neuroprotection against ischemic injury both *in vivo* and *in vitro*. The roles of other PcG proteins in the neuronal response to ischemia are not known. The objective of this study was to investigate the possible involvement of PcG proteins of the PRC2 complex in neuroprotection, with a focus on the Enhancer of Zest Homolog-2 (EZH2), a methyltransferase that trimethylates histone H3 at lysine 27. We also examined whether or not enhanced neuroprotection can be achieved by simultaneous regulation of both PRC1 and PRC2 group proteins. All experiments were performed on differentiated neuroblastoma NS20Y cells. Over-expression of recombinant EZH2 protein was achieved by transfection. Inhibition of EZH2 activity was achieved by incubating cells with EPZ6438, an EZH2-specific inhibitor. Simulated ischemia was modeled by oxygen-glucose deprivation (OGD). OGD-induced cell injury was determined by LDH assay. Potassium currents were recorded by whole-cell patch clamp. Levels of mRNA or protein of interest were determined by qPCR, Western blotting and immunocytochemistry, respectively. Interaction of EZH2 protein with the promoter region of its potential target gene was examined by chromatin immunoprecipitation (ChIP) followed by PCR analyses. Results to date have shown that over-expression of EZH2 reduced OGD-induced injury, whereas inhibition of EZH2 activity exacerbated the injury. Whole cell potassium currents were significantly decreased with EZH2 over-expression and increased with EZH2 inhibition. An interaction between EZH2 and the promoter region of potassium channel gene Kcna1, either directly or indirectly, was evidenced by the results of ChIP assay. Further, a compounding repressive effect on whole cell potassium currents was observed when histone

modification activities of both EZH2 and BMI1 - a PRC1 protein, were inhibited, when compared with that of inhibition of EZH2 or BMI1 alone. In conclusion, our results support a role of EZH2 in neuroprotection against ischemic injury in cultured neuronal cells. The underlying mechanisms may involve PcG protein-mediated suppression of potassium channel proteins. Further studies will aim at the involvement of additional PcG proteins and a possible, broader involvement of potassium channel proteins.

Disclosures: P. Sharma: None. **T. Leng:** None. **Z. Xiong:** None. **Z. Yang:** None. **R. Simon:** None. **A. Zhou:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.26/G4

Topic: C.08. Ischemia

Support: NRF 2005-0093836

the Korean Health Technology R&D Project HI14C1913

Asan Institute 2015-624

Title: Pyruvate and cilostazol protect cultured rat cortical pericytes against tissue plasminogen activator (tPA)-induced cell death

Authors: *H. KIM[1], T.-Y. KIM[1], Y. YOON[2], J.-Y. KOH[3];
[1]Asan Inst. For Life Sci., Seoul, Korea, Republic of; [2]Dept. of Ophthalmology, Asan Med. Ctr., Seoul, Korea, Republic of; [3]Dept. of Neurology, Asan Med. Ctr., Seoul, Korea, Republic of

Abstract: Since even a brief ischemia can cause permanent brain damage, rapid restoration of blood flow is critical to limiting damage. Although intravenous tPA during the acute stage is the treatment of choice for achieving reperfusion, this treatment is sometimes associated with brain hemorrhage. Agents that reduce tPA-related bleeding risk may help expand its therapeutic window. This study assessed whether zinc dyshomeostasis underlies the toxic effect of tPA on brain vascular pericytes; whether pyruvate, an inhibitor of zinc toxicity, protects pericytes against tPA-induced cell death; and whether cilostazol, which protects pericytes against tPA-induced cell death, affects zinc dyshomeostasis associated with tPA toxicity. Cultured pericytes from newborn rat brains were treated with 10-200 μg/ml tPA for 24 h, inducing cell death in a concentration-dependent manner. tPA-induced cell death was preceded by increases in intracellular free zinc levels, and was substantially attenuated by TPEN. Pyruvate completely blocked direct zinc toxicity and tPA-induced pericyte cell death. Both cAMP and cilostazol, a PDE3 inhibitor that attenuates tPA-induced pericyte cell death *in vitro* and tPA-induced brain

hemorrhage *in vivo*, reduced zinc- and tPA-induced pericyte cell death, suggesting that zinc dyshomeostasis may be targeted by cilostazol in tPA toxicity. These findings show that tPA-induced pericyte cell death may involve zinc dyshomeostasis, and that pyruvate and cilostazol attenuate tPA-induced cell death by reducing the toxic cascade triggered by zinc dyshomeostasis. Since pyruvate is an endogenous metabolite and cilostazol is an FDA-approved drug, *in vivo* testing of both as protectors against tPA-induced brain hemorrhage may be warranted.

Disclosures: H. Kim: None. **T. Kim:** None. **Y. Yoon:** None. **J. Koh:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.27/G5

Topic: C.08. Ischemia

Support: NIH/NINDS 1R01 NS073832

Arthur McDowell general assistance

Title: Mechanisms of neuroprotection elicited by polycomb group protein scmh1

Authors: *A. KEY, P. SHARMA, Z. YANG, A. MCDOWELL, A. ZHOU;
Neurosci. Inst., Morehouse Sch. of Med., Atlanta, GA

Abstract: Background Polycomb group (PcG) proteins are repressive, transcriptional regulatory proteins controlling the expression of a broad spectrum of genes by means of histone protein modifications. They function in multi-protein complexes. SCMH1 is a PcG protein associated with polycomb repressive complex (PRC) I. It, together with several other PcG proteins of PRC-I such as BMI1 and RING1B, have been shown to exhibit neuroprotective roles against ischemic injury, both in ischemic mouse brains *in vivo* and in cultured neuronal cells *in vitro* (Stapels et al., 2010, Sci Sig xxx). Little is known for the molecular mechanisms that underlie SCMH1-mediated neuroprotection. Unlike RING1B or BMI1 proteins of PRC-I, SCMH1 does not possess any histone modification activity. Hence, we speculate that SCMH1 may exert its role primarily by mediating protein-protein/protein-DNA interaction and/or sustaining the integrity of PRC-I. The objective of this study was to characterize changes in neuronal cells that can be induced by changes in the level of SCMH1 protein and to identify proteins that may interact with SCMH1, as our first step in dissecting SCMH1-mediated neuroprotective mechanisms. Methods Over- or under-expression of SCMH1 protein was achieved by transfection of recombinant SCMH1 cDNA or siRNA against SCMH1, respectively, into neuronal NS20Y cells. For comparison, over-expression of several other PcG proteins was also included. Cellular levels and subcellular distribution of SCMH1 protein was determined by Western blot and immunocytochemistry analyses, respectively. Protein interaction with SCMH1 under normal or

ischemic conditions was determined by immunoprecipitation using an anti-SCMH1 antibody followed by Western blotting of the known PRC-I protein and mass spectrometry analysis of the entire interactive complex. Results A profound change in the morphology of NS20Y cells was observed in cells with SCMH1 over-expression. When examined under light microscrope, SCMH1-over-expressing cells presented more processes when compared with that in wildtype cells. Such a change was not seen in NS20Y cells over-expressing other PcG proteins of PRC-I: BMI1 and RING1B and PRC-I protein EZH2. Western blot analysis of cellular proteins that were immunoprecipitated with an anti-SCMH1 antibody resulted in a positive identification of PRC-1 protein RING1B. This supports the association of SCMH1 with PRC-I in neuronal cells. On-going mass spectrometry analysis focus on other immunoprecipitated proteins extracted from cells subjected to different ischemic conditions, and is expected to reveal protein networks that may be regulated by SCMH1.

Disclosures: A. Key: None. **P. Sharma:** None. **Z. Yang:** None. **A. McDowell:** None. **A. Zhou:** None.

Poster

307. Ischemia: Molecular Mechanisms and Neuroprotection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 307.28/G6

Topic: C.08. Ischemia

Title: A transcriptional program underlying neuronal survival

Authors: *B. MAINO[1], S. PAPARONE[2], C. SEVERINI[3], V. D'AGATA[4], M. CIOTTI[2], P. CALISSANO[3], S. CAVALLARO[2];
[1]ISN-CNR, Catania, Italy; [2]Inst. of Neurolog. Science, Italian Res. Council, Catania, Italy; [3]Inst. of Neurolog. Science, Italian Res. Council, Roma, Italy; [4]Dept. di Scienze Bio-Mediche, Univ. of Catania, Catania, Italy

Abstract: A shift of the delicate balance between apoptosis and survival-inducing signals determines the fate of neurons during the development of the central nervous system and in its homeostasis throughout adulthood. Both apoptosis- and survival-signaling pathways converge into the nucleus and regulate gene expression. Although the transcriptional program of neuronal apoptosis has been elucidated, the transcriptional program underlying neuronal survival is still unknown. We conducted whole-genome expression profiling to decipher the transcriptional regulatory elements controlling the apoptotic/survival switch in cerebellar granule neurons following the induction of apoptosis by serum and potassium deprivation and the rescue by different neurotrophic factors. Although acting through different upstream signaling pathways, the survival effects of neurotrophic factors converged to common transcriptional cascades. In addition to revealing the existence of a previously unknown transcriptional program underlying

neuronal survival, our analysis identified common transcriptional changes intersecting neuronal apoptosis and survival, forming the basis for further functional analyses and pharmacological investigation.

Disclosures: **B. Maino:** None. **S. Paparone:** None. **C. Severini:** None. **V. D'Agata:** None. **M. Ciotti:** None. **P. Calissano:** None. **S. Cavallaro:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.01/G7

Topic: C.08. Ischemia

Support: Ochsner Clinic Foundation

Title: Profiling plasma miRNA in intracerebral hemorrhage of small and large volume size

Authors: R. C. MARTINEZ[1], A. VARMA[2], I. O. IWUCHUKWU[3], *D. NGUYEN[4]; [1]Neurol., Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA; [2]Kasturba Med. Sch., Manipal, India; [3]Ochsner Med. Ctr., New Orleans, LA 70121, LA; [4]Neurosurg., Ochsner Hlth. Syst., New Orleans, LA

Abstract: Introduction: The potential role of miRNA as biomarkers in the pathophysiology of cerebral injury has been increasingly recognized. Studies have suggested that the expression patterns of miRNA change in relation to cerebrovascular diseases such as ischemic and hemorrhagic stroke. However, knowledge on the association of plasma miRNA with hemorrhagic stroke is still lacking. It is known that in intracerebral hemorrhage (ICH) hematoma volume >30 ml is associated with worse clinical outcome. We aim to determine the difference in circulating miRNA expression in plasma of patients with acute spontaneous ICH stratified by volumes (< 30ml and > 30ml). Methods: Ten patients with acute spontaneous ICH were recruited and categorized into small volume (<30ml) and large volume (>30ml) hematoma. Blood samples were collected within 48 hours of admission. Total RNA containing miRNA were extracted from 200ul of plasma samples using RNeasy Plasma miRNA Mini Kit. The cDNA and real-time PCR were carried out using the miRCURY LNA Universal RT and ExiLENT Sybr Green kits and run on an ABI 7500 real-time PCR. Ten candidate miRNAs were selected from our screen of 752 miRNAs using the Exiqon platform: miR-23a-3p, miR-24-3p, miR-30c-5p, miR-103a, miR-125b-5p, miR-183, miR-190-5p, miR-338-3p, miR-374b, and hsa-let7a. Results: We report here for the first time the presence of miRNA in brain pathogenesis associated with ICH as well as differentiation between small and large hematoma volume. MiR-103a was detected in high abundance in all ten ICH patients. On the other hand, miR-338 and miR-190 were found at low levels. The miR-125b was found at moderate but consistent levels across all samples.

Comparison between the large and small hematoma volume revealed that all patients with <30cc hematoma volume had detectable levels of miR-23a-3p, but also in some large volume patients. Notably, miR-183 was only detected in the ICH with the greatest volume (80ml). Conclusion: These results do not demonstrate distinct differences in expression of miRNA in plasma between <30ml and >30ml hematoma. Expressions of miR-338 and miR-125b were consistent across all samples which suggest that they may be potential biomarkers of spontaneous ICH, regardless of hematoma size. Additionally, we may imply that miR-183 is sensitive for very large volume hematoma.

Disclosures: R.C. Martinez: None. **A. Varma:** None. **I.O. Iwuchukwu:** None. **D. Nguyen:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.02/G8

Topic: C.08. Ischemia

Support: Foundation for Physical Therapy/ Promotion of Doctoral Studies Fellowship

Minnesota Medical Foundation

National Center for Research Resources to the University of Minnesota CTSI 1UL1RR033183

Title: Test-retest reliability of transcranial magnetic stimulation measures in stroke

Authors: *J. M. CASSIDY[1], H. CHU[2], J. R. CAREY[3];
[1]Neurol., Univ. of California, Irvine, Irvine, CA; [2]Div. of Biostatistics, Sch. of Publ. Hlth., [3]Program in Physical Therapy, Dept. of Physical Med. and Rehabil., Univ. of Minnesota, Minneapolis, MN

Abstract: Objective: Determining the efficacy of non-invasive brain stimulation (NIBS) requires reliable transcranial magnetic stimulation (TMS) measures for probing corticomotor excitability. Despite the introduction of TMS nearly 30 years ago, there remains a lack of investigation concerning the reliability of TMS measures in stroke. The primary objective of this study was to examine the test-retest reliability of several intracortical and interhemispheric TMS measures in individuals with chronic stroke. **Methods:** Participants completed two sessions of TMS testing separated by approximately 24 hours. Test-retest reliability of ipsilesional resting motor threshold (RMT), cortical silent period (CSP) duration, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and interhemispheric inhibition (IHI) was assessed using two-way, mixed intraclass correlation coefficients ($ICC_{(3,k)}$). Secondary analyses

included the computation of standard error of measurement and minimal detectable change values for each TMS measure. We also examined the influence of motor-evoked potential (MEP) analysis methodology (i.e. MEP amplitude vs. MEP area) on TMS paired-pulse measurement reliability by visually inspecting corresponding 95% confidence intervals for ICCs. **Results:** Eleven individuals (3 females; average age = 66 ± 9.4 years) with chronic stroke completed the study. Participants tolerated all procedures. CSP and log-transformed ICF and RMT measures demonstrated good to excellent reliability (ICCs ≥ 0.75). SICI and IHI measures derived from both MEP amplitude and MEP area measures demonstrated poor reliability (ICC < 0.50). The overlap of confidence intervals across all paired-pulse TMS measures suggested nonsignificant differences in measurement reliability between TMS measures calculated from MEP amplitude vs. area. **Conclusions:** Considerable differences in reliability exist amongst TMS measures regardless of the MEP method of analysis. Results from this study will strengthen future NIBS studies with regard to corticomotor excitability measurement selection and will ultimately help address present-day concerns of intra- and inter-individual variability in NIBS response.

Disclosures: J.M. Cassidy: None. **H. Chu:** None. **J.R. Carey:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.03/G9

Topic: C.08. Ischemia

Title: Feasibility of robotic assessment in cynomolgus macaques following middle cerebral artery occlusion (MCAO)

Authors: *S. V. OLESOVSKY[1], J. Y. NASHED[1], J. Z. WANG[1], T. ST. AMAND[1], D. J. COOK[1,2];
[1]Ctr. for Neurosci. Studies, [2]Dept. of Surgery, Div. of Neurosurg., Queen's Univ., Kingston, ON, Canada

Abstract: Recent studies have highlighted the efficacy of non-human primate(NHPs) stroke models for preclinical, translational prior to initiating human trials. However, the relevance of NHP neurobehaviour in modeling human clinical grading has not been reliably demonstrated. Robotic assessment tools represent a quantitative, reliable and reproducible means to assess neurobehaviour following stroke in both humans and NHPs. Here we investigate the feasibility of robotics to explore neurobehavioural deficits in NHPs following MCAO. To investigate the feasibility of robotic assessments in NHPs following MCAO we employed the KINARM exoskeleton, which permits elbow and shoulder movements in the horizontal plane. Two Cynomolgus macaques underwent transient MCAO for 90min and recovered for 30days. Following the 30 day recovery period NHPs were trained to perform unassisted reaching

movements with both their affected and non-affected limbs in the KINARM exoskeleton. NHPs made reaching movements from a centrally positioned start target to 1 of 8 peripheral targets uniformly distributed around the start target. Each target required differing amount of elbow and shoulder movement. Following MCA stroke, the NHPs became proficient as the centre-out reaching task. In both animals we observed inter-limb differences. Reaching movements with the non-affected arms were straight with bell-shaped velocity profiles. In contrast, the reaching movements with the affected arm of both animals were highly variable, compared to the non-affected arm, and reaching movements often contained multiple velocity peaks. Finally, initial direction errors were far more prevalent in the affected arm (>83%) compared to the non-affected arm (<18%). These initial results qualitatively match the performance of human stroke subjects, suggesting that robotic neurobehavioural assessment in NHPs with stroke is feasible and have translational relevance to subsequent human studies.

Disclosures: S.V. Olesovsky: None. **J.Y. Nashed:** None. **J.Z. Wang:** None. **T. St. Amand:** None. **D.J. Cook:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.04/G10

Topic: C.08. Ischemia

Support: NIH Grant R01 NR-013625

NIH Grant R01 NR-014669

Title: Altered hippocampal resting-state functional connectivity in patients with heart failure

Authors: *B. PARK[1], M. A. WOO[2], P. M. MACEY[2], G. C. FONAROW[3], R. M. HARPER[4], R. KUMAR[1,5,6];
[1]Anesthesiol., [2]Sch. of Nursing, [3]Div. of Cardiol., [4]Neurobio., [5]Radiological Sci., [6]Bioengineering, Univ. of California At Los Angeles, Los Angeles, CA

Abstract: Heart failure (HF) is accompanied by damage to brain areas responsible for mood, cognition, and autonomic regulation, which are independent predictors for morbidity and mortality in the condition. Injury to affective and cognitive regulatory regions, including the hippocampus, may lead to symptoms of depression and deficient memory through altered spontaneous functional connections to other brain areas. However, how the hippocampus in HF is connected abnormally with other brain regions during resting conditions, and what functional deficits accompany these impaired connections are unclear. Our aim was to assess hippocampal functional connectivity (FC) with other brain regions in HF subjects, compared to healthy controls. We acquired resting-state functional MRI data from 17 hemodynamically-optimized

HF (age, 54.4±8.1 years; body-mass-index (BMI), 29.4±5.7 kg/m2; 12 male; left ventricular ejection fraction, 28.0±7.0%) and 45 control subjects (age, 50.9±7.6 years; BMI, 25.1±3.8 kg/m2; 30 male) using a 3.0-Tesla MRI scanner. Data were processed using SPM8 with standard procedures, canonical nuisance signals were removed by regression, and data were band-pass filtered. Using left and right hippocampal seed regions, we calculated individual correlation maps between each seed area and all other brain voxels, and converted those values into z-scored maps. We compared z-scored maps voxel-by-voxel between HF and control subjects using analysis of covariance (covariates; age and gender; $P<0.05$; cluster-corrected). The left hippocampus showed decreased FC in HF with the right superior frontal gyrus and left inferior parietal lobule, while increased FC in HF appeared with the left middle cingulate cortex, left superior temporal gyrus, left calcarine cortex, and right cerebellum. The right hippocampus in HF showed decreased FC with the bilateral thalamus, right middle frontal gyrus, and left inferior occipital gyrus, compared to controls. However, increased FC from the right hippocampus in HF appeared within the left supplementary motor area, vermis, and left middle frontal gyrus. Resting state functional connections in HF are altered between the hippocampus and other autonomic, affective, and cognitive regulatory areas, and the changes are lateralized predominantly to left rostral brain areas. The reorganization of function, especially with cerebellar, cingulate, and frontal regions, may reflect compensatory means to restore capabilities of damaged areas, and likely result from structural brain injury reported-earlier in HF.

Disclosures: B. Park: None. **M.A. Woo:** None. **P.M. Macey:** None. **G.C. Fonarow:** None. **R.M. Harper:** None. **R. Kumar:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.05/G11

Topic: C.08. Ischemia

Support: Ochsner Clinic Foundation

Title: Circulating miRNA profile in cerebrospinal fluid and plasma of patients with spontaneous intracerebral hemorrhage

Authors: A. VARMA[1], *R. C. MARTINEZ[3], I. IWUCHUKWU[2], D. NGUYEN[2]; [2]Neuro-Critical Care, [1]Ochsner Med. Ctr., New Orleans, LA; [3]Neurol., Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Background and Objectives: Intracerebral hemorrhage (ICH) in patients with stroke carries a significant morbidity and mortality. miRNA, which are non-coding RNA, have been identified in tissues and recently in plasma (circulating miRNA). Access to brain tissue is often

challenging, hence cerebrospinal fluid (CSF) serves as a surrogate for brain tissue. It is hypothesized that differences in circulating miRNA compared to miRNA in CSF may reflect the local events of injured brain tissue. In this study, we described the expression pattern of CSF and circulating miRNA in patients with spontaneous ICH. Methods: Total RNA containing miRNAs were isolated from 200 ul of CSF (n=5) and plasma (n=3) using serum/plasma kit (Exiqon). All RNA samples were within acceptable limits as analyzed using miRNA QC panel. For miRNA profiling, the Exiqon Human miRNome panel I and II containing 752 well-characterized miRNAs were used and run using a CFX384 thermal cycler. Cycle threshold data were obtained using the regression method. Quality control assessment, global mean normalization, and differential miRNA expression were analyzed using the unpaired T-test and GenEx software (Exiqon). Similarity of miRNA expression patterns between CSF and plasma samples were analyzed by hierarchal clustering and heat maps using default settings. Results: Quality control assessment using RNA and cDNA spike-in controls were within acceptable values. Hemolysis analysis for possible cellular derived miRNA contamination was negative. Statistical analysis for differentially expressed miRNAs found 166 miRNAs that were significantly expressed ($p<0.01$), of these 110 miRNAs expressed at greater than 3- fold higher in CSF, and 42 miRNAs that were expressed at greater than 3-fold higher in plasma. Hierarchal clustering and heat maps showed miRNA expression patterns in CSF tightly cluster and are distinct from miRNA expression in plasma. Conclusion: We demonstrated that miRNAs are present in CSF and plasma of patients with ICH. miRNAs are potentially useful noninvasive biomarkers for diagnosis of disease, its complications and outcome. Our current focus is to confirm and correlate miRNAs expression to clinical outcome based on ICH blood volume classifier.

Disclosures: **A. Varma:** None. **R.C. Martinez:** None. **I. Iwuchukwu:** None. **D. Nguyen:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.06/G12

Topic: C.08. Ischemia

Support: NIH Grant 5R01HD061117

NINDS Grant P30 NS048056

American Heart Association - 14PRE19610010

Title: Hemodynamic lag: prevalence after stroke and effects on functional connectivity

Authors: *J. S. SIEGEL, L. E. RAMSEY, A. Z. SNYDER, G. L. SHULMAN, M. CORBETTA;
Neurol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Stroke can cause a disruption to the brain's vascular supply, not only within but also outside of areas of infarction. In addition to reduced blood flow, altered hemodynamics have been reported. We investigated temporal delays (lag) in resting state functional magnetic resonance imaging signals in 130 stroke patients who were scanned 2 weeks, 3 months and 12 months post stroke onset. 30 controls were scanned twice at an interval of 3 months. Hemodynamic lag was determined using cross-correlation with the global gray matter signal. Behavioral performance in multiple domains was assessed in all patients. Regional cerebral blood flow and carotid patency were assessed in subsets of the cohort using arterial spin labeling and carotid Doppler ultrasonography. Severe hemodynamic lag was observed in 30% of stroke patients sub-acutely. Approximately 10% of patients showed lag at 1-year post-stroke. Hemodynamic lag corresponded to gross aberrancy in functional connectivity measures. Hemodynamic lag also correlated with performance deficits in multiple domains and with local and global perfusion deficit. Correcting for lag partially normalized abnormalities in measured functional connectivity. However, post-stroke FC-behavior relationships in the motor and attention systems persisted even after hemodynamic delays were corrected. Resting state fMRI can reliably identify areas of hemodynamic delay following stroke. Our data reveal that hemodynamic delay is common sub-acutely and of clinical importance. Such changes grossly alter measures of functional connectivity and should be accounted for in any future resting state fMRI analyses in patients with vascular pathology.

Disclosures: J.S. Siegel: None. **L.E. Ramsey:** None. **A.Z. Snyder:** None. **G.L. Shulman:** None. **M. Corbetta:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.07/G13

Topic: C.08. Ischemia

Support: NIH Grant R01 NR-013625

NIH Grant R01 NR-014669

Title: Impaired resting-state insular brain functional connectivity in heart failure

Authors: *R. VIG[1], B. PARK[1], M. A. WOO[2], G. C. FONAROW[3], R. M. HARPER[4], R. KUMAR[1,5,6];

[1]Anesthesiol., [2]Sch. of Nursing, [3]Div. of Cardiol., [4]Neurobio., [5]Radiological Sci., [6]Bioengineering, UCLA, Los Angeles, CA

Abstract: Brain structural and metabolic deficits appear in multiple areas in heart failure (HF) subjects, including the insular cortices that may contribute to deficient autonomic regulation by altering spontaneous functional connections with other brain areas. Our aim was to assess insular functional connectivity (FC) with other brain regions in HF subjects at rest, compared to healthy controls. We acquired resting-state functional MRI data from 17 hemodynamically-optimized HF (age, 54.4±8.1 years; body-mass-index (BMI), 29.4±5.7 kg/m2; 12 male; left ventricular ejection fraction, 28.0±7.0%) and 45 control subjects (age, 50.9±7.6 years; BMI, 25.1±3.8 kg/m2; 30 male), using a 3.0-Tesla MRI scanner. Data were processed using SPM8 software with standard procedures, effects of gray matter, white matter, cerebrospinal fluid, and 6 rigid motions and their derivatives were removed by regression, and data were low band-pass filtered. Using the left and right insular seed regions, we calculated individual correlation maps between each seed area and all other brain voxels, and converted those values into z-scored maps. We compared z-scored maps voxel-by-voxel between HF and control subjects using analysis of covariance (covariates; age and gender; $P<0.05$; cluster-corrected). No significant differences in age or gender appeared, but BMI values were significantly higher in HF over controls. The left insula showed decreased FC in HF with the left supplementary motor area and left cerebellum, while increased FC in HF appeared with the left inferior frontal gyrus over control subjects. The right insula in HF showed decreased FC with the right superior parietal gyrus and right postcentral gyrus, and increased FC appeared in the left inferior frontal gyrus, left temporal pole, and left cerebellum over control subjects. Sensory integrative roles of the right insula would be affected with the reduced parietal cortex FC, and expected, considering the specific insular injury in HF. The enhanced FC with the left side may compensate for the loss in right-side autonomic influences. The insula FC changes demonstrate the disparate roles for the left and right structures, and show the complex reorganization that occurs with projections to other brain areas from this somatosensory/autonomic integrative region in HF.

Disclosures: R. Vig: None. **B. Park:** None. **M.A. Woo:** None. **G.C. Fonarow:** None. **R.M. Harper:** None. **R. Kumar:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.08/G14

Topic: C.08. Ischemia

Support: NIH Grant R01AG033570

Predoctoral NIH Training Grant T32 HL007692-25

Title: Changes in microglia and neural stem cell characteristics after exposure to hypoxia

Authors: ***M. K. TOBIN**[1,2,3], J. A. BONDS[1,3], E. SZILAGYI[4], A. M. BARTHOLOMEW[4,5], D. A. PELLIGRINO[6], O. LAZAROV[1];
[1]Anat. and Cell Biol., [2]Med. Scientist Training Program, [3]Grad. Program in Neurosci., [4]Surgery, [5]Bioengineering, [6]Anesthesiol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Post-ischemia inflammation has been a target of stroke therapy for some time but no treatments have successfully made it beyond clinical trials. These therapies have largely been targeted at reducing the overall inflammatory response rather than trying to modify how inflammatory cells respond to stroke. Furthermore, neurogenic mechanisms fail to repair damaged brain tissue following stroke despite an acute proliferative burst of neural stem cells (NSC). To start to address these issues, we first examined the effect of hypoxia on the phenotype of microglia and neural progenitor cells. While grown under oxygen-glucose deprivation (OGD) conditions, NSCs exhibit an increase in proliferation after 24 hours. However, this proliferative capacity rapidly declines back to normal levels beyond 24 hours. Additionally, mRNA expression of BDNF, NT3, NGF, GDNF, and IGF-1 are all significantly increased in NSCs following 24 hours of OGD which could account for the initial proliferative burst. Next, because of their ability to interconvert microglia from an inflammatory to a regenerative phenotype, we utilized mesenchymal stem cells (MSC) to investigate whether the pro-inflammatory phenotype of microglia following hypoxia can be changed. For this purpose, microglia were grown in conditioned media from both naïve MSCs (nMSC) as well as interferon-γ (IFN-γ)-activated MSCs (aMSC). Interestingly, microglia treated by aMSC conditioned media exhibited a reduction in expression of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α up to 48 hours in culture and had increased expression of the pro-regenerative cytokines IL-10 and IL-4 up to 7 days in culture. Taken together, these results suggest that neural progenitor cells may be beneficial for neuronal survival and repair following an ischemic event, and that MSCs may have the capacity to promote a pro-regenerative microglial phenotype that can enhance brain repair following ischemia.

Disclosures: **M.K. Tobin:** None. **J.A. Bonds:** None. **E. Szilagyi:** None. **A.M. Bartholomew:** None. **D.A. Pelligrino:** None. **O. Lazarov:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.09/G15

Topic: C.08. Ischemia

Support: Ontario Brain Institute (OBI)

NeuroDevNet (NDN)

Title: Effects of the constraint-induced movement therapy (cimt) and neural precursor cell (npc) transplantation in the corpus callosum of the hemiplegic mouse model

Authors: *P. RUMAJOGEE[1], S. ALTAMENTOVA[1], D. VAN DER KOOY[2], M. G. FEHLINGS[1];
[1]Genet. and Develop., Univ. Hlth. Network, Toronto, ON, Canada; [2]Dept. of Mol. Genet., Inst. of Med. Science, Univ. of Toronto, Toronto, ON, Canada

Abstract: BACKGROUND: Cerebral palsy (CP) is the most common paediatric neurodevelopmental physical disability, with a prevalence of 2.3/1000 births, causing severe motor and developmental disturbances. It has been suggested that Constraint-Induced Movement Therapy (CIMT) could provide functional benefit by stimulating neural cell generation from endogenous neural precursor cell (NPC). However, the underlying mechanisms of CIMT and optimal timing/mode of application remain poorly understood. METHODS: We use the Hypoxic-Ischemic model (HI) model which involve the Right Common Carotid Artery Occlusion (RCAO) of P7 C57Bl/6 mice pups, followed by Hypoxia (8% oxygen for 45 minutes). The project on has 3 major aspects: 1) Regeneration, which will focus on the effects of NPC transplantation in the corpus callosum (CC), known to be impacted early in the course of demyelinating conditions. 2) Rehabilitation, which will focus on the effects CIMT (via Botox injections). 3) A third aspect will also investigate the potential of CIMT and transplanted NPCs. RESULTS: Our preliminary results support the use of the HI model as an interesting model for Hemiplegic CP. We have shown: A) Differences between Injured and Control animals, as well as within injured groups. B) A good development and integration of NPCs in CC, as well as some co-localisation with Olig4 and NeuN positive cells. C) A potential role of NPCs in functional recovery. D) An improved the Botox injection protocol, which will allow a more efficient CIMT. CONCLUSION: This work suggests that the variability of injury observed after the exact same experimental procedure can recapitulate various aspects of clinical CP phenotype, which might be useful when creating translational treatment strategies. Further investigation is needed in order to decipher the mechanisms of CIMT and a potential role of NPCs to improve this therapy.

Disclosures: P. Rumajogee: None. **S. Altamentova:** None. **D. Van Der Kooy:** None. **M.G. Fehlings:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.10/G16

Topic: C.08. Ischemia

Support: NIH Grant 1R01NS071956-01A1

NIH Grant 1R21NS089851-01

James and Esther King Biomedical Research Foundation 1KG01-33966

Title: Intravenously transplanted human bone marrow mesenchymal stem cells preferentially migrate to spleen and abrogate splenic inflammatory response in a chronic ischemic stroke model

Authors: *S. A. ACOSTA, N. TAJIRI, J. HOOVER, Y. KANEKO, C. V. BORLONGAN; Universty of South Florida, Tampa, FL

Abstract: Adult stem cell therapy is an experimental stroke treatment. Here, we assessed homing and anti-inflammatory effects of bone marrow stromal cells (hBMSCs) in chronic stroke. At 60 days post-stroke, animals received intravenous hBMSCs (4X106 labeled or non-labeled cells) or vehicle (saline). A sham surgery group served as additional control. *In vivo* imaging was conducted between 1 hour and 11 days post-transplantation, followed by histological examination. Labeled hBMSCs migrated to spleen which emitted significantly higher fluorescent signal across all time points, especially during the first hour, but were only modestly detected in the head region at the 12 hours and 11 days, compared to non-labeled hBMSCs and vehicle-infused stroke animals, or sham (p's < 0.05). At 11 days post-transplantation, *ex vivo* imaging confirmed preferential hBMSC migration to the spleen over the brain. H&E staining revealed significant 15% and 30% reductions in striatal infarct and peri-infarct area, respectively, and a trend of rescue against neuronal loss in the hippocampus. Unbiased stereology showed significant 75% and 60% decrements in MHCII-activated inflammatory cells in gray and white matter, and a 43% diminution in TNF-α cell density in the spleen of hBMSC- transplanted stroke animals compared to vehicle-infused stroke animals (p's < 0.05). Human antigen immunostaining revealed 0.03% hBMSCs survived in spleen and only 0.0007% in brain. hBMSC migration to spleen, but not brain, inversely correlated with reduced cerebral infarct, peri-infarct, and inflammation. hBMSC transplantation exerts therapeutic effects on chronic stroke possibly by abrogating the inflammation-plagued secondary cell death.

Disclosures: S.A. Acosta: None. **N. Tajiri:** None. **J. Hoover:** None. **Y. Kaneko:** None. **C.V. Borlongan:** None.

Poster

308. Ischemia: Human and Translational Studies and Cell-Based Therapies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 308.11/G17

Topic: C.08. Ischemia

Support: Asterias Biotherapeutics, Inc grant

Title: hESC-Oligodendrocyte Precursor Cell transplantation after white matter stroke enhances recovery

Authors: *I. L. LLORENTE[1], N. C. MANLEY[2], C. C. CASE[2], E. WIRTH, III[2], S. T. CARMICHAEL[1];
[1]Neurol., Univ. of California , Los Angeles, Los Angeles, CA; [2]Asterias Biotherapeutics, Inc., Menlo Park, CA

Abstract: Subcortical white matter stroke (WMS) constitutes up to 25% of all stroke subtypes and is the second leading cause of dementia. We have developed a new subcortical WMS model in NSG mice with a large infarct area that mimics the larger white matter lesions seen in moderate to advanced human white matter ischemia or vascular dementia. While there is no therapy for white matter stroke, cell transplantation is emerging as a viable therapy to restore neurological function. WMS has a very different pattern of cellular injury than occurs in traditional stroke models. The neural elements damaged in WMS are: oligodendrocytes, oligodendrocyte progenitor cells (OPCs), astrocytes, and axons. Human embryonic stem cell-derived oligodendrocyte progenitor cells (hESC-OPCs) provide an attractive candidate for a WMS therapy. These cells are in a lineage that matches the most damaged cellular element in white matter ischemia: cells in the oligodendrocyte lineage. We determined the tissue outcomes in myelination patterns, reactive astrocytosis, microglial/macrophage responses, MRI appearance of WMS, and the optimum injection site for hESC-OPC transplantation. Transplant of hESC-OPCs at subacute time points (7 days after stroke) outside of the ischemic stroke produced widespread migration of these cells throughout subcortical white matter. This resulted in increased myelination within the damaged white matter, reduced measures of reactive astrocytosis and inflammation. Transplantation of cells into the stroke site resulted in larger infarct area, the same level of astrocytosis and inflammatory responses within the damaged white matter and less measure of myelination compared to hESC-OPCs transplanted outside of the infarct area. MRI imaging of white matter stroke after hESC-OPC transplantation outside of the lesion showed reduction in the hyperintensities that characterize this damage in both the mouse model and in humans. Behavioral evaluation of recovery is ongoing. These studies suggest that hESC-OPC transplantation in moderate to advanced white matter stroke, as seen in vascular dementia, may provide a therapy to promote white matter repair and recovery. Supported by a research grant from Asterias Biotherapeutics, which was not involved in research analysis or data interpretation.

Disclosures: I.L. Llorente: None. **N.C. Manley:** A. Employment/Salary (full or part-time):; Asterias Biotherapeutics, Inc. **C.C. case:** A. Employment/Salary (full or part-time):; Asterias Biotherapeutics, Inc. **E. Wirth:** A. Employment/Salary (full or part-time):; Asterias Biotherapeutics, Inc. **S.T. Carmichael:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Asterias Biotherapuetics, Inc.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.01/G18

Topic: C.10. Trauma

Support: NIH Grant NS061839 to A.I.F.

NIH Grant NR012686 to S.G.D.

Title: miR-711 up-regulation induces neuronal cell death after traumatic brain injury

Authors: *B. SABIRZHANOV[1], B. A. STOICA[2], Z. ZHAO[2], D. J. LOANE[2], J. WU[2], S. DORSEY[2], A. I. FADEN[2];
[1]Dept. of Anesthesiol., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Anesthesiol., Univ. of Maryland Sch. of Medicine, Baltimore, Baltimore, MD

Abstract: Traumatic brain injury (TBI) is a leading cause of mortality and disability. MicroRNAs (miRs) are small noncoding RNAs that negatively regulate gene expression at post-transcriptional level and may be key modulators of neuronal apoptosis, yet their role in secondary injury after TBI remains largely unexplored. Changes in miRs after controlled cortical impact (CCI) in mice were examined during the first 72h using miR arrays and qPCR. One selected miR (711) was examined with regard its regulation and relation to cell death; effects of miR-711 modulation were evaluated after CCI and using *in vitro* cell death models of primary cortical neurons. Levels of miR-711 were increased in the cortex early after TBI and *in vitro* models through rapid up-regulation of miR-711 transcription (pri-miR-711) rather than catabolism. Increases coincided with down-regulation of the pro-survival protein Akt, a predicted target of miR-711, with sequential activation of FoxO3a/GSK3α/β, pro-apoptotic BH3-only molecules PUMA and Bim, and mitochondrial release of cytochrome c and AIF. miR-711 and Akt (mRNA) co-immunoprecipitated with the RNA-induced silencing complex (RISC). A miR-711 hairpin inhibitor attenuated the apoptotic mechanisms and decreased neuronal death in an Akt-dependent manner. Conversely, a miR-711 mimic enhanced neuronal apoptosis. Central administration of the miR-711 hairpin inhibitor after TBI increased Akt expression and attenuated apoptotic pathways. Treatment reduced cortical lesion volume, neuronal cell loss in cortex and hippocampus, and long-term neurological dysfunction. miR-711 changes contribute to neuronal cell death after TBI, in part by inhibiting Akt, and may serve as a novel therapeutic target.

Disclosures: B. Sabirzhanov: None. **B.A. Stoica:** None. **Z. Zhao:** None. **D.J. Loane:** None. **J. Wu:** None. **S. Dorsey:** None. **A.I. Faden:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.02/G19

Topic: C.10. Trauma

Support: Grants from National Natural Science Foundation of China (No. 81201461)

the Natural Science Foundation of Chongqing, China (No.CSTC2012jjA10107)

Title: Adenosine A2A receptor blockade alleviates memory dysfunction caused by traumatic brain injury via ROCK2 inhibition

Authors: Y.-L. NING, Z.-A. ZHAO, N. YANG, Y. PENG, R.-P. XIONG, X. CHEN, Y. ZHAO, P. LI, *Y. ZHOU;
Res. Inst. Surg and Daping Hosp. ,TMMU, Chongqing, China

Abstract: Memory dysfunction is one of the most serious neuropsychiatric disorders of traumatic brain injury (TBI) because its long-lasting effects prevent survivors resuming normal civilian life. However, the mechanisms are poorly understood and there is no treatment available. Adenosine A2A (A2AR) is one of the key modulators of cognitive function and brain injury in the central nervous system (CNS). In this study, the effects of adenosine A2AR on TBI-induced memory dysfunction and the underlying mechanisms were investigated. Moderate controlled cortical impact (CCI) was used to perform a TBI model in adult male c57BL/6 mice. At 1 week post-CCI, mice exhibited impaired spatial reference memory and working memory in a Morris Water Maze paradigm, accompanied by hippocampal neuronal degeneration including neuronal loss and neurite dystrophy. Activation of A2AR with selective A2AR agonist CGS21680 significantly exacerbated CCI-induced memory dysfunction and the hippocampal neuropathological damages, which was rescued by selective A2AR antagonist ZM241385 and Rho-associated coil kinase (ROCK) inhibitor fasudil. Further investigation of primary mice hippocampal neurons demonstrated that A2AR activation decreased length and brunches of dendrites, suppressed synapsin1 expression and increased Tau protein S262 phosphorylation during Okadaic acid (OA)-induced toxicity *in vitro*. Moreover, fasudil rescued all of the above deteriorated neurite damages. Western blot analysis demonstrated that CGS21680 increased, while ZM241385 decreased the expression of ROCK2. Together, A2AR blockade alleviated memory dysfunction following TBI via ROCK2 inhibition, suggesting A2AR and ROCK pathway as potential therapeutic targets for the treatment of TBI-induced cognitive impairment.

Disclosures: **Y. Ning:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Grants from National Natural Science Foundation of China (No. 81201461), the Natural Science Foundation of Chongqing, China (No.CSTC2012jjA10107). **Z. Zhao:** None. **N. Yang:** None. **Y. Peng:** None. **R. Xiong:** None. **X. Chen:** None. **Y. Zhao:** None. **P. Li:** None. **Y. Zhou:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.03/G20

Topic: C.10. Trauma

Support: NINDS RO1 NS077767

Title: Activated neutrophils are modifiers of barrier disruption after pediatric traumatic brain injury

Authors: *A. TRIVEDI, K. TERCOVICH, L. J. NOBLE-HAEUSSLEIN;
Univ. of California, San Francisco, San Francisco, CA

Abstract: Blood brain barrier (BBB) compromise is a key pathophysiological component of secondary damage after traumatic brain injury (TBI). We have previously shown by immunohistological outcomes that controlled cortical impact to the developing murine brain at postnatal day 21 (p 21), an age that approximates the toddler aged child, results in a unique inflammatory signature, characterized by the prolonged recruitment of neutrophils (up to 14 days post injury) to cortical and subcortical brain regions. Moreover, barrier disruption, as determined by measures of vasogenic edema, is attenuated in neutrophil elastase knockout mice, suggesting that the activational state of neutrophils is a determinant of barrier disruption. Here, we confirm the prolonged time course of neutrophil recruitment in the injured developing brain by flow cytometry and provide the first evidence that recruitment involves distinct subsets of neutrophils. Neutrophils are activated via the downstream signaling pathway dependent on spleen tyrosine kinase (Syk). Conditional knockouts of Syk (sykf/f MRP8-cre+) along with cogenic littermates (sykf/f) were used to study the dependency of barrier disruption on activated neutrophils by two complementary assays, Evan's blue albumin (EBA) extravasation as measured by colorimetry and by histological measures of fluorescence intensity resulting from extravasation of different molecular weight dextrans. Barrier disruption to EBA was attenuated in sykf/f MRP8-cre+ compared to brain-injured sykf/f. Fluorescently tagged dextrans corresponding to the molecular weights of albumin (MW 70kD) and fibrinogen (MW 500kD) revealed profound leakage in the ipsilateral hemisphere. While the 70kD dextran was distributed more broadly throughout the injured hemisphere, the 500kD dextran was prominent surrounding vascular structures. Importantly, barrier disruption to these tracers was significantly reduced in the sykf/f MRP8-cre+ mice. Together, these findings support the extended recruitment of subsets of neutrophils to the injured developing brain. While the long-term consequences of this extended recruitment remain unclear, we find that the activational state of neutrophils is a key determinant of barrier disruption and as such may influence subsequent recovery processes.

Disclosures: A. Trivedi: None. **K. Tercovich:** None. **L.J. Noble-Haeusslein:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.04/G21

Topic: C.10. Trauma

Support: NJCBIR grant CBIR14IRG019

NJCBIR multi-investigator grant CBIR12MIG011

National Institutes of Health under Ruth L. Kirschstein National Research Service Award T32 GM8339 from the NIGMS

Title: The role of mTOR/Akt pathway in recovery of neural electrophysiology in an *in vitro* model of traumatic brain injury

Authors: *P. SWIATKOWSKI[1,2], B. L. FIRESTEIN[2];
[2]Cell Biol. & Neurosci., [1]Rutgers Univ., Piscataway, NJ

Abstract: Traumatic brain injury (TBI) affects approximately 1.7 billion people each year and is the leading cause of death in people under 45 years of age in the United States. TBI is primarily caused by deformations of the brain tissue due to mechanical trauma, followed by rapid release of glutamate and subsequent excitotoxicity (secondary damage). Additionally, the early phase of secondary injury has been found to cause loss of dendritic spines and formation of varicosities along dendrites and axons. Several groups have associated spine loss with behavioral deficits following brain injury, and further evidence shows that aiding neurons in spine reemergence drastically improves functional recovery. The mTOR/Akt pathway has been implicated in the modulation and regulation of synaptic strength, synaptic activity, maturation, and axon regeneration, all of which might contribute to functional recovery of neurons subjected to TBI. To mimic mechanical and secondary injury as a result of TBI in rat cortical neurons, we grew these cells *in vitro* on either silastic membranes or glass coverslips and subjected them to stretch or NMDA -induced injury, respectively. In this study, we addressed electrophysiological properties, such as field potentials and spontaneous EPSCs, of neurons subjected to two types of injury and potential recovery following mTOR/Akt pathway manipulation. To manipulate these pathways, we used several compounds, such as the PTEN inhibitor bpV, the Akt inhibitor MK2206 and the mTORC1 inhibitor RAD001 (a rapamycin analog), and assessed their effects on neural electrophysiology following injury. We predict that further investigation of the role of mTOR/Akt pathway in TBI will contribute to improved functional recovery of neurons and subsequently improve lives of affected individuals. This work is supported by NJCBIR grant CBIR14IRG019 and NJCBIR multi-investigator grant CBIR12MIG011 and National Institutes of Health under Ruth L. Kirschstein National Research Service Award T32 GM8339 from the NIGMS.

Disclosures: **P. Swiatkowski:** None. **B.L. Firestein:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.05/G22

Topic: C.10. Trauma

Title: N-acetyl-seryl-aspartyl-lysyl-proline improves functional recovery in rats after traumatic brain injury

Authors: ***Y. ZHANG**[1], M. CHOPP[2,3], Y. MENG[1], L. ZHANG[2], Z. ZHANG[2], A. MAHMOOD[1], Y. XIONG[1];
[1]Neurosurg., [2]Neurol., Henry Ford Hosp., Detroit, MI; [3]Physics, Oakland Univ., Rochester, MI

Abstract: N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is a naturally occurring multifunctional tetrapeptide. The pharmacokinetics and metabolism of AcSDKP have been well established, and there is no apparent toxicity in rodents. AcSDKP can readily cross the blood brain barrier which poses significant challenges to the permeation of neuroprotective agents. This study was designed to test the hypothesis that AcSDKP treatment initiated 1 hour post injury provides neuroprotection and improves functional recovery in rats after traumatic brain injury (TBI). TBI was induced by controlled cortical impact over the left parietal cortex. Young adult male Wistar rats with TBI were randomly divided into the following groups: (1) Vehicle group (0.01N acetic acid); (2) AcSDKP (0.8 mg/kg/day). AcSDKP or vehicle was administered subcutaneously starting at 1 hour post injury and continuously for 3 days through an osmotic minipump. Sensorimotor function and spatial learning were assessed using a modified neurological severity score and Morris water maze tests, respectively. Animals were sacrificed 35 days after injury and brain sections processed to assess lesion volume, hippocampal cell loss, angiogenesis, neurogenesis and dendritic spine remodeling after AcSDKP treatment. Compared to the vehicle treatment, AcSDKP treatment initiated 1 hour post injury significantly improved sensorimotor functional recovery (Days 14-35, $p<0.05$) and spatial learning (Days 32-35, $p<0.05$), reduced cortical lesion volume by 30% ($p<0.05$) and hippocampal cell loss, enhanced neurogenesis and the number of dendritic spine in the injured hippocampus ($p<0.05$). AcSDKP treatment initiated 1 hour post injury provides neuroprotection and neurorestoration after TBI, indicating that this small tetrapeptide has promising therapeutic potential for treatment of TBI. These data warrant further investigation of the optimal dose and therapeutic window of AcSDKP treatment for TBI and the associated underlying mechanisms.

Disclosures: **Y. Zhang:** None. **M. Chopp:** None. **Y. Meng:** None. **L. Zhang:** None. **Z. Zhang:** None. **A. Mahmood:** None. **Y. Xiong:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.06/G23

Topic: C.10. Trauma

Title: Asparagus racemosus exhibits anti-post traumatic stress disorder (PTSD)-like effect through 5HT1A mediated mechanism in experimental rats

Authors: *R. VIG, D. GARABADU, P. ADITYA, S. KRISHNAMURTHY;
Indian Inst. of Technol. (BHU), Varanasi, India

Abstract: Post-traumatic stress disorder (PTSD) is considered as one of the most prevalent anxiety disorder in worldwide. Though several synthetic drugs are prescribed for PTSD, their use is limited because of their adverse effects. Asparagus racemosus (AR) is considered as adaptogen according to Ayurvedic medicine. It exhibits anxiolytic and anti-stress activities in both preclinical and clinical studies. Therefore, the present study investigated the therapeutic effect of standardized methanolic root extract of AR (MAR) in an experimental animal model of PTSD. On day-2 (D-2) of the experimental schedule, male rats were subjected to induce PTSD-like behaviors using modified stress re-stress (SRS) protocol of 2 hr restraint and 20 min forced-swim test (FST) followed by halothane anesthesia. The rats were exposed to re-stress (FST) on D-8 and at six day intervals on D-14, D-20, D-26 and D-32. The rats were treated with MAR (50, 100 and 200 mg/kg; p.o.) and standard drug, sertraline (STL; 10.0 mg/kg; p.o.) from D-8 to D-32. MAR (200 mg/kg) attenuated SRS-induced depressive-like behavior in terms of decrease in immobility period in rats during FST. The highest dose of MAR also reduced anxiety in elevated plus maze (EPM) test in PTSD-like animals in terms of increase in the percentage entries and time spent in open arms. Further, MAR (200 mg/kg) improved SRS-induced cognitive deficit such as loss in spatial recognition memory in Y-maze test in terms of increase in the percentage in entries into novel arm compared to known arm. The SRS-induced decrease in the plasma corticosterone level in rodents were reversed with the repeated treatment of MAR (200 mg/kg), suggesting the fact that MAR may modulates SRS-induced alteration in hypothalamic-pituitary-adrenal cortex-axis function in rats. Furthermore, MAR (200 mg/kg) reversed the SRS-induced alteration in serotonergic activity in amygdala. Subsequently, the effect of MAR (200 mg/kg) on 5-HT1A-mediated mechanism in PTSD pathophysiological condition was evaluated. The 5-HT1A receptor antagonist (WAY-100635; 1.0 mg/kg, i.p.) attenuated the MAR (200 mg/kg)-induced improvement in depression, anxiety and cognitive impairment in PTSD-like rats in FST, EPM and Y-maze paradigms respectively. These observations emphasize the fact that MAR (200 mg/kg) may exert anti-PTSD-like effect in animals through 5-HT1A-mediated mechanism. Hence, AR could be a potential therapeutic option in the management of PTSD.

Disclosures: R. Vig: None. **D. Garabadu:** None. **P. Aditya:** None. **S. Krishnamurthy:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.07/G24

Topic: C.10. Trauma

Support: MOST 103-2321-B-038-003

NSC 101-2321-B-038-004

NSC 102-2321-B-038-004

NSC 101-2632-B-038-001-MY3

Title: Glucose-dependent insulinotropic polypeptide reduces sensorimotor impairments after traumatic brain injury in rats

Authors: *Y.-W. YU[1,2], T.-H. HSIEH[2,3,5], J.-H. LAI[3,4], K.-Y. CHEN[2,3], J.-W. LIN[4], B. J. HOFFER[2,6], N. H. GREIG[7], Y.-H. CHIANG[2,3,4];
[2]Grad. Inst. of Neural Regenerative Med., [3]Ctr. for Neurotrauma and Neuroregeneration, [4]Dept. of Surgery, [1]Taipei Med. Univ., Taipei, Taiwan; [5]Grad. Inst. of Rehabil. Sci., Chang Gung Univ., Taoyuan, Taiwan; [6]Dept. of Neurosurg., Case Western Reserve Univ. Sch. of Med., Cleveland, OH; [7]Drug Design & Develop. Section, NIH, Baltimore, MD

Abstract: Mild traumatic brain injury (mTBI) is a majority of TBI survivor and may lead to various physical, cognitive, emotional and psychological-related disorders following mTBI. Until now, there is no efficient intervention to manage the development of mTBI. Earlier studies showed that glucagon-like peptide-1 (GLP-1) has potential neuroprotective effects. Here, we identified its sister incretin, glucose-dependent insulinotropic polypeptide (GIP), as the novel approach mTBI. GIP was delivered by i.p. implanted with ALZET micro-osmotic pumps. Beam walking and adhesive removal test were assessed for sensorimotor functions in rats with mTBI. Glial fibrillary acidic protein (GFAP), bone marrow tyrosine kinase gene in chromosome X (BMX)/epithelial, endothelial tyrosine kinase (Etk) and beta-amyloid precursor protein (APP) were applied as the molecular markers by immunohistochemistry (IHC) staining or western blotting (WB). The results show that the sensorimotor deficits were reduced after two weeks treatment of GIP. Based on the results of IHC and WB, the sensorimotor recovery could involve with the astrocytic activation, inflammative response and axonal injury. These findings suggest that the GIP may have beneficial effects in the management of secondary events following mTBI in rodent models. Future preclinical studies will be needed to further identify the mechanism of action, leading to more effective drug therapies in human.

Disclosures: **Y. Yu:** None. **T. Hsieh:** None. **J. Lai:** None. **K. Chen:** None. **J. Lin:** None. **B.J. Hoffer:** None. **N.H. Greig:** None. **Y. Chiang:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.08/G25

Topic: C.10. Trauma

Support: NIH grants NS060005, HD069620 and NS084967 (PI: Anthony E. Kline, Ph.D.)

Title: A combined regimen of environmental enrichment and citalopram improves attentional set-shifting after brain trauma

Authors: *C. O. BONDI, M. J. LAPORTE, H. M. TENNANT, J. P. CHENG, A. E. KLINE; Safar Ctr. for Resuscitation Res., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Introduction Traumatic brain injury (TBI) models in the laboratory have been associated through the literature with declines in long-term learning and memory, although the types of behavioral tests typically do not focus on complex attention impairments related to the frontal lobe, which are common in most brain injuries. Specifically, executive function and cognitive flexibility represent sophisticated brain capabilities to use environmental feedback to "unlearn" a previously valid set of rules, filter out distractions and switch gears to new contingencies. Previously, we demonstrated that a controlled cortical impact (CCI) injury produced significant impairments in executive function and cognitive flexibility in the attentional set-shifting test (AST), a complex cognitive paradigm analogous to the Wisconsin Card Sorting Test, which is used to measure strategy-switching deficits in patients with frontal lobe damage, TBI, and psychiatric disorders. Hypothesis Exposure to clinically relevant therapies post injury, such as environmental enrichment (EE) and the antidepressant drug, citalopram, will attenuate cognitive performance deficits on AST when provided alone, but especially when given in combination. Methods Isoflurane-anesthetized male rats were subjected to CCI injury (2.8 mm cortical deformation depth at 4 m/s) or sham injury. Groups of rats from both surgical conditions were exposed EE housing, an endorsed animal model of rehabilitation, while daily injections of citalopram, a treatment known to alleviate depressive-like symptoms and improve cognition in humans, were also provided alone or in combination with EE. Rats were tested on the AST at four weeks post-surgery. The AST involves a series of increasingly difficult discriminative tasks to obtain food reward, including simple and compound discriminations, stimulus reversals, and intra- and extradimensional (ED) shifts. Results EE exposure provided significant cognitive recovery after injury, although performance may further benefit from the combined therapy with citalopram, as findings indicate. Conclusions Exposure to EE housing and daily citalopram administration provided the most cognitive recovery on AST after injury, which supported the hypothesis. Significance The combined treatment in this study aims to reflect simultaneous rehabilitation and pharmacological treatments given to patients in a clinical setting. Future

studies will continue to investigate in more detail the ideal cognitive recovery timeline and specific brain pathways and mechanisms involved in restoring higher function after TBI.

Disclosures: C.O. Bondi: None. **M.J. LaPorte:** None. **H.M. Tennant:** None. **J.P. Cheng:** None. **A.E. Kline:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.09/G26

Topic: C.10. Trauma

Support: NIH R01 grants NS060005 and HD069620

Title: Delayed and abbreviated environmental enrichment after experimental traumatic brain injury increases hippocampal neurogenesis

Authors: N. LAJUD[1], J. P. CHENG[2], C. O. BONDI[2], *A. E. KLINE[2];
[1]Ctr. de Investigación Biomédica de Michoacán, Inst. Mexicano del Seguro Social, San José la Huerta, Mexico; [2]Phys Med. & Rehab, Psych, Safar Ctr. Resuscitation Res., Univ. Pittsburgh, Pittsburgh, PA

Abstract: Continuous environmental enrichment (EE) exposure improves neurobehavioral outcome after experimental traumatic brain injury (TBI). One likely mechanism for the benefit is increased neurogenesis. Delayed and abbreviated EE, which is more akin to clinical rehabilitation in terms of timing, also enhances neurobehavioral recovery comparably to early and continuous exposure after TBI; however, its effect on hippocampal neurogenesis is unknown. *The aim of this study was to test the hypothesis that delayed and abbreviated EE is sufficiently robust to induce hippocampal neuroplasticity after TBI*. Anesthetized adult male rats received a controlled cortical impact (2.8 mm depth at 4 m/sec) and were randomly assigned to either standard housing (TBI+STD), continuous EE(TBI+EE), or delayed and abbreviated EE (TBI+EE, 3 day delay, 6 hr day). BrdU (500 mg/kg) was provided twice per day for 3 days and then sacrificed 10 days later. The brains were cut on a freezing microtome at 40 μm and immunostained for BrdU or triple immunofluorescence for BrdU, DCX and NeuN. Continuous EE lead to a 91% ($p \leq 0.05$) increase in BrdU labeled nuclei density in the subgranular zone of the dentate gyrus when compared to STD. Abbreviated EE resulted in a 156% increase ($p \leq 0.01$) relative to STD. Triple immunofluorescence showed no differences in the percentage of BrdU/DCX or BrdU/NeuN double labeled cells among the groups; however in the continuous EE group, DCX positive cells displayed larger ramifications when compared to abbreviated EE. In conclusion, abbreviated EE with a 3 day delay effectively induced hippocampal neurogenesis

after TBI, which supports the hypothesis. These findings elucidate a possible mechanism for the benefits observed with both continuous and delayed-and-abbreviated EE.

Disclosures: **N. Lajud:** None. **J.P. Cheng:** None. **C.O. Bondi:** None. **A.E. Kline:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.10/G27

Topic: C.10. Trauma

Support: NIH Grant R01N5086422

Title: Time-dependent mechanisms of striatal stimulation for enhanced neural recovery following traumatic brain injury

Authors: A. HUGUENARD, *H. KATNANI, E. ESKANDAR;
Dept. of Neurosurg., Massachusetts Gen. Hosp., Boston, MA

Abstract: In a previous publication (Katnani et al.; Society for Neuroscience Abstracts 2014) we found that unilateral deep brain stimulation (DBS) in the nucleus accumbens (NAc) in the sub-acute phase of traumatic brain injury can enhance functional recovery and induce widespread cellular adaptation. Specifically, histology following behavioral testing revealed an increase in neuronal precursor cells in prefrontal cortex and restoration of corticostriatal synaptic density for animals that received stimulation. To expand on this finding, we next investigated the time-dependent processes of gross cellular adaptation induced by stimulation during recovery. Mice underwent a unilateral controlled cortical impact injury and were implanted with sham or stimulating electrodes targeted to the NAc. At 2, 5, 7, or 14 days after stimulation, brains were extracted for immunohistochemical analysis. Four categories of markers were studied: cellular proliferation (BrdU, NeuN, DoubleCortin, and GFAP), cell migration and plasticity (Synaptophysin and GAP43), cell injury, scarring, or death (Caspase 3, Iba-1, and FluoroJade-B), and finally cellular activation (c-Fos). Analysis was focused in the hippocampus, anterior rostral migrating stream, striatum, subventricular zone, and perilesional area, revealing an increase in markers of neuroproliferation, neuroplasticity, and neuroprotection for animals treated with DBS. These findings begin to elucidate the underlying dynamics of gross cellular mechanisms induced by striatal DBS following brain injury.

Disclosures: **A. Huguenard:** None. **H. Katnani:** None. **E. Eskandar:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.11/G28

Topic: C.10. Trauma

Support: University of Utah Seed Grant

Title: A hemostatic, tissue adhesive with immunomodulatory properties

Authors: *J. L. SKOUSEN, M. POLEI, P. A. TRESCO;
Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: The search for the ideal hemostat, sealant and adhesive for use in neurosurgical applications continues. Despite recent advances, the only agents approved for use across this spectrum are fibrin-based products, which result in neuroinflammation, fibrous tissue deposition and scar formation where they are applied. This is especially problematic as neuroinflammation and scar formation can result in aberrant electrical conduction at the application site and can isolate implants from functioning in concert with adjacent tissue. What is needed is a new tissue adhesive that rapidly clots blood, can fill large wound cavities, and provides immunomodulatory signals to promote constructive wound healing with reduced scarring. To accomplish this we have developed an adhesive composed of extracellular matrix (ECM) and aldehyde-modified chondroitin sulfate (CS-aldehyde). ECM was chosen as the adhesive's foundation due to its innate ability to initiate and promote the clotting cascade. In addition to ECM's hemostatic properties, a number of studies have shown that ECM collected from decellularized tissues is also immunomodulatory and reduces inflammatory sequela by directing reactive macrophages toward a pro-healing rather than a classically activated, pro-inflammatory phenotype. Multiple formulations of the adhesive were prepared by varying the ratio of either cell-type-specific or commercial ECM to the CS-aldehyde crosslinker. To examine the adhesive and wound filling abilities of the resulting formulations, we applied them to surgical incisions made on freshly isolated rat brains. We found that the adhesives were capable of adhering together surgically separated tissues as well as filling large wound cavities. Current work is focused on further characterizing the mechanical, hemostatic and immunomodulatory properties of the various adhesive formulations before testing in a controlled cortical brain impact injury model in rodents.

Disclosures: J.L. Skousen: None. **M. Polei:** None. **P.A. Tresco:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.12/G29

Topic: C.10. Trauma

Support: NIH-5T32HD040686-14

VA I01RX001127

NIH-NS060672

NIH-NS40125

The Pittsburgh Foundation

Title: Administration of lithium improves neurotransmission and increases vesicular docking proteins in the striatum after traumatic brain injury

Authors: *S. W. CARLSON[1], A. DESANA[2], E. MADHA[2], H. Q. YAN[1], C. E. DIXON[1]; [1]Neurosurg. and VA Pittsburgh Healthcare Syst., [2]Neurosurg., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) impairs neuronal function and can culminate in lasting cognitive impairment. While impaired striatal dopamine release is reported after experimental TBI, little is known about the mechanisms underlying this consequence. Our previous work suggests that reductions in proteins comprising the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, the machinery facilitating vesicular fusion, may contribute to altered synaptic vesicle properties and impaired neurotransmission. Cysteine-string protein α (CSPα) is an important chaperone protein that facilitates SNARE complex formation and is increased in response to lithium treatment. The objective of this study was to evaluate the effect of lithium administration on CSPα abundance and neurotransmission in the striatum after controlled cortical impact (CCI). Methods: Sprague-Dawley rats received CCI (2.7mm) or sham injury. Animals were treated with vehicle or 1.0mmol/kg/ml lithium chloride daily (i.p. injection) for 1wk, beginning 5 minutes post-injury. The brains were dissected at 1wk post-injury and processed for immunoblotting of CSPα (n=3-4/group). At 1wk post-injury, CCI-injured and sham-injured rats were subjected to microdialysis and evoked dopamine release was measured by high-performance liquid chromatography (n=4-5/group). Results: CCI results in a 40% reduction in CSPα abundance in the striatum following injury. Treatment with lithium after CCI increased CSPα in the striatum by 15%, compared to vehicle treatment. CCI was associated with a significant reduction in peak dopamine release following high-potassium stimulated release ($p < 0.05$). The peak dopamine levels with post-traumatic treatment of lithium were not different from sham injury ($p=0.75$). Conclusions: These findings provide the first evidence of altered SNARE protein abundance in the striatum after CCI. We demonstrate for the first time that lithium improves neurotransmission following TBI. These findings suggest that treatment with lithium after TBI may increase the abundance of important proteins that facilitate neurotransmitter release into the synaptic cleft.

Disclosures: S.W. Carlson: None. **A. DeSana:** None. **E. Madha:** None. **H.Q. Yan:** None. **C.E. Dixon:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.13/G30

Topic: C.10. Trauma

Support: DoD grant W81XWH-10-1-0578 to GF

LF supported by Medical Student Summer Research Program

Title: Effects of early glucocorticoid antagonism or late progesterone agonism on the psychological symptoms of mild traumatic brain injury in male and female rats

Authors: *L. C. FOX, D. R. DAVIES, G. M. PALMER, J. L. SCHOLL, M. J. WATT, G. L. FORSTER;
Univ. of South Dakota, Vermillion, SD

Abstract: Mild traumatic brain injuries (mild TBIs) comprise three-quarters of total TBIs in the United States each year, and often occur in settings of stress (warfare, sports, etc.). Their psychological symptoms can have major impacts on wellbeing. These symptoms most often include generalized anxiety and posttraumatic stress-like behaviors. Stress at the time of injury is known to influence severity of post-mild TBI symptoms, and neuroactive steroids may play a role in preventing these effects. Prior to injury, blockade of glucocorticoids may lessen the impact of stress on damaged neurons. Progesterone delivery after injury has shown promise in restoring cognitive function in moderate-to-severe TBI, but its effect on psychological symptoms of mild TBI has not been evaluated. Using our validated model of social defeat immediately prior to weight-drop induced mild TBI, adult male and female Sprague-Dawley rats (9wks old) received either injury or sham surgery. Rats were treated either with mifepristone (20 mg/kg) 40min before impact, or progesterone (4 mg/kg) 3 hours after impact and once per day for 5 days. Female rats were intact and naturally cycling. Female social defeat was achieved with a 3-week isolation paradigm for non-lactating residents, who then showed similar levels of aggression toward intruding females as did male resident rats toward intruding experimental males. Animals were evaluated for anxiety 8 days after injury in the elevated plus maze (EPM). Contextual fear conditioning was assessed in the same animals 11 days after injury, using a foot-shock paradigm with an initial conditioning day and 3 days of extinction testing. Sex differences were assessed, as well as differences across estrus phases. We show that glucocorticoid antagonism prior to injury prevents anxiety symptoms from arising, restoring mild TBI animals to control levels of time spent in the open arms of the EPM. Female rats were less anxious than males overall, and displayed less freezing behavior as a whole than did males during fear conditioning trials. While early targeting of glucocorticoid receptors holds promise in preventing affective symptoms seen after mild TBI, later targeting of progesterone receptors after injury has already occurred may achieve similar benefit.

Disclosures: **L.C. Fox:** None. **D.R. Davies:** None. **G.M. Palmer:** None. **J.L. Scholl:** None. **M.J. Watt:** None. **G.L. Forster:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.14/G31

Topic: C.10. Trauma

Support: NFL Charities Foundation

Indiana Department of Health Spinal Cord and Brain Injury Fund

Mizzou Advantage

University of Missouri Department of Pathology & Anatomical Sciences Research Fund

Title: Water-soluble gelatinase inhibitor O-phosphate prodrug and its metabolite p-hydroxy SB-3CT ameliorate motor functions against brain damage after severe traumatic injury in mice

Authors: *M. R. JUAREZ[1,4], Z. CHEN[2,4], M. LEE[5], B. TOMLINSON[2,4,3], M. GOOYIT[5], R. NIZAM[2,4], D. HESEK[5], B. BOGGESS[5], V. A. SCHROEDER[5], W. R. WOLTER[6], M. A. SUCKOW[6], J. CUI[2,7,4], S. MOBASHERY[5], M. CHANG[5], Z. GU[2,7,4];
[2]Dept. of Pathology & Anatom. Sci., [3]MS in Pathology Program, Univ. of Missouri Grad. Sch., [1]Univ. of Missouri - Columbia, Columbia, MO; [4]Ctr. for Translational Neurosci., Columbia, MO; [5]Dept. of Chem. and Biochem., Univ. of Notre Dame, Notre Dame, IN; [6]Dept. of Biol. Sci., Freimann Life Sci. Ctr. , Univ. of Notre Dame,, Notre Dame, IN; [7]Univ. of Missouri Grad. Sch., Ctr. for Botanical Interaction Studies, Columbia, MO

Abstract: Traumatic brain injury (TBI) affects 1.7 million individuals in the United States, resulting in 52,000 annual deaths and accounting for 30% of all injury deaths. While the primary causes of TBI are immensely broad, studies point to matrix metalloproteinases (MMPs), particularly MMP-9 as a key factor in the pathogenesis of TBI secondary injury. It includes generation of reactive oxygen species and pro-inflammatory cytokines, resulting in neuroinflammation, edema, blood-brain barrier (BBB) breakdown, oxidative stress, mitochondrial dysfunction, and apoptosis. SB-3CT (1) is a potent and selective gelatinase (MMP-2 and MMP-9) inhibitor, which effectively reduces brain damage after severe TBI in mice. However, SB-3CT is poorly water-soluble and is metabolized primarily to p-hydroxy SB-3CT (2), a more potent inhibitor than SB-3CT. In the present study, we examined the effects of the O-phosphate prodrug (3) of p-hydroxy SB-3CT on motor functions and histological changes in C57Bl/6J mice with TBI. Prodrug 3, an inactive MMP inhibitor, has enhanced water solubility by more than 2000-fold and is readily hydrolyzed to the active metabolite 2 in human blood.

Pharmacokinetics and brain distribution studies in mice showed that metabolite 2 crossed the blood-brain barrier (BBB) and achieved therapeutic concentrations in the brain. Mice were divided into three groups: sham, vehicle, and prodrug 3/metabolite 2. Prodrug 3 was administered intravenously at 7.8 mg/kg at 30 minutes after TBI, followed by subcutaneous injections of p-hydroxy SB-3CT at 25 mg/kg 1 hour after TBI and daily for the next 3 or 6 days. Simple Neuroassessment of Asymmetric Impairment (SNAP) and beam-walking tests were performed to assess neurological impairment; cresyl violet staining using the stereological technique was used to assess brain lesion. SNAP results for the sham were the lowest, as expected, followed in increasing order by 7-day treatment, 3-day treatment and vehicle. While foot faults for sham and 3-day treatment stayed relatively constant over time, the average number of foot faults in 7-day treatment mice decreased, indicating greater motor control. Our results suggest prodrug 3/metabolite 2 treatment over seven days improves motor functions and decreases neuronal damage. These findings indicate that selective inhibition of MMP-9 by a water-soluble thiirane inhibitor is a promising therapy for treatment of severe TBI.

Disclosures: **M.R. Juarez:** None. **Z. Chen:** None. **M. Lee:** None. **B. Tomlinson:** None. **M. Gooyit:** None. **R. Nizam:** None. **D. Hesek:** None. **B. Boggess:** None. **V.A. Schroeder:** None. **W.R. Wolter:** None. **M.A. Suckow:** None. **J. Cui:** Other; Department of Pathology and Anatomical Sciences, University of Missouri School of Medicine, Columbia, Missouri 65212, USA, Center for Translational Neuroscience, Center for Botanical Interaction Studies, University of Missouri, Columbia, MO, USA. **S. Mobashery:** Other; Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA. **M. Chang:** Other; Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA. **Z. Gu:** Other; Department of Pathology and Anatomical Sciences, University of Missouri School of Medicine, Columbia, Missouri 65212, USA, Center for Translational Neuroscience, Center for Botanical Interaction Studies, University of Missouri, Columbia, MO, USA.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.15/G32

Topic: C.10. Trauma

Support: DCMRP #DR080470

NIH P20MD006988

Title: Dietary omega-3 polyunsaturated fatty acids ameliorate PTSD-like behaviors while improving hippocampal morphology following mild traumatic brain injury in rats

Authors: *J. D. FIGUEROA[1], T. HEERS[1], P. KALYAN-MASIH[1], J. VEGA-TORRES[1], E. KINNEY-LANG[2], M. DE LEON[1], A. OBENAUS[2];
[1]Ctr. for Hlth. Disparities and Mol. Med., [2]Pediatrics, Loma Linda Univ. Sch. of Med., Loma Linda, CA

Abstract: Despite accumulating evidence showing an elevated risk for post-traumatic stress disorder (PTSD) following mild traumatic brain injury (mTBI), there has been little progress in developing effective secondary preventive interventions. We have shown that long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) exhibit therapeutic potential for the treatment and prevention of the neurological deficits associated with neurotrauma. However, studies showing the effects of n-3 PUFAs to ameliorate the PTSD-like brain and behaviors are lacking. The present study: (1) examines the efficacy of n-3 PUFAs to ameliorate PTSD-like behaviors following mild controlled cortical impact (CCI) TBI in adult rats and (2) evaluates the impact of dietary n-3 PUFAs on hippocampal volumes following chronic mTBI. Rats were fed with either control chow or chow enriched with n-3 PUFAs (750 mg/kg/day) for 4 weeks before being subjected to a single mTBI. We used a controlled cortical impact (CCI; 4 mm diameter tip, 0.5 mm depth, 6.0 m/s speed, 200 ms dwell) to induce mTBI. Animals were allowed to survive for 4 weeks after trauma and the brains collected for high-resolution magnetic resonance imaging (MRI). We found that consumption of n-3 PUFA significantly reduced functional deficits in locomotion (CatWalk gait analyses), sensorimotor gating (pre-pulse inhibition; PPI) and anxiety-like behaviors following mTBI. Magnetic resonance imaging (MRI) demonstrated that dietary n-3 PUFAs preserved hippocampal volume when compared to animals fed with the control chow ($p < 0.05$). Immunofluorescence revealed an increased number of neurons in the hippocampus of animals fed n-3 PUFA diets when compared to controls. Collectively, our study demonstrates that the neurobehavioral responses associated with PTSD-like behaviors are ameliorated by dietary n-3 PUFAs following mTBI.

Disclosures: J.D. Figueroa: None. **T. Heers:** None. **P. Kalyan-Masih:** None. **J. Vega-Torres:** None. **E. Kinney-Lang:** None. **M. De Leon:** None. **A. Obenaus:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.16/G33

Topic: C.10. Trauma

Support: The Miami Project to Cure Paralysis

NIH/NINDS NS056072

NIH/NINDS NS069721

Title: Selective PDE4B inhibition reverses chronic memory impairments following traumatic brain injury

Authors: *D. J. TITUS[1], C. FURONES[1], W. D. DIETRICH[1], M. E. GURENY[2], C. M. ATKINS[1];
[1]Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Tetra Discovery Partners, Grand Rapids, MI

Abstract: Development of learning and memory impairments after traumatic brain injury (TBI) is a common consequence for TBI survivors. However, there are no effective treatments to improve TBI-induced cognitive impairments. A pan-phosphodiesterase 4 (PDE4) inhibitor was shown to reduce cognitive deficits after TBI, but clinical development is hampered by unwanted side effects. Our previous study found that TBI induces expression of the subfamily isoform PDE4B2. Developing more subtype-selective inhibitors would greatly improve clinical translation to TBI survivors. In the present study, we hypothesized that treating animals with a subtype-specific PDE4B inhibitor could reverse the cognitive deficits induced by TBI. To test this hypothesis, adult male Sprague Dawley rats received sham surgery or moderate parasagittal fluid-percussion brain injury (2 atm). After 3 mos of recovery, animals were treated with a selective PDE4B inhibitor 2-(4-{[2-(5-chlorothiophen-2-yl)-5-ethyl-6-methylpyrimidin-4-yl] amino} phenyl) acetic acid (A33) (0.3mg/kg, i.p) or vehicle. At 30 min after treatment, animals received cue and contextual fear conditioning, water maze training, and spatial working memory assessment. A33 treatment significantly reversed the TBI-induced deficits in the cue and contextual fear conditioning and water maze retention. To further understand the underlying cellular mechanisms of these memory impairments, we examined long-term potentiation (LTP) in area CA1 of the hippocampus at 3 mos after TBI or sham surgery. There was a significant reduction in basal synaptic transmission and impaired LTP in hippocampal slices from TBI animals as compared to sham surgery animals. A33 treatment (0.3 μM) significantly reduce the deficits in basal synaptic transmission and rescued expression of LTP. These results suggest that a subtype-selective PDE4B inhibitor may be a potential therapeutic to reverse chronic cognitive dysfunction and deficits in hippocampal synaptic plasticity following TBI.

Disclosures: D.J. Titus: None. **C. Furones:** None. **W.D. Dietrich:** None. **M.E. Gureny:** None. **C.M. Atkins:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.17/G34

Topic: C.10. Trauma

Support: PAPIIT IG201014

Title: Effect of repetitive transcranial magnetic stimulation (rTMS) in traumatic brain injury (TBI) of rats

Authors: L. VERDUGO[1], A. HERNANDEZ-CHAVEZ[1], E. HERNANDEZ -LOPEZ[1], A. GARCIA-ESPONOZA[1], F. ESTRADA-ROJO[1], M. MARTINEZ-VARGAS[1], *L. NAVARRO[2], A. PEREZ-ARREDONDO[1];
[1]Physiol., [2]UNAM Fac Med., Mexico DF 04510, Mexico

Abstract: TBI is one of the leading causes of death and disability worldwide. In addition to the damage caused at the moment of injury, it causes secondary injury, but also induces neuroprotective mechanisms; which may even be promoted through external strategies. We analyzed the effect of rTMS in the behavioral recovery and brain histological analysis of rats with TBI. We used 70 male Wistar rats, maintained in vivarium conditions for 7 days, some of them habituated in movement restriction boxes for 15 min daily. Afterward, they were divided into 6 group; G1: control without movement restriction; G2: control with movement restriction and sham rTMS; G3: control with movement restriction and rTMS, G4 to G6 were anesthetized and subjected to TBI using a standardized device; G4: TBI without movement restriction; G5, TBI and movement restriction plus sham rTMS; G6: TBI and movement restriction plus rTMS. rTMS (2Hz) or restriction was applied for 15 min daily for 7 days. Body weight, water and food intake, and motor skill behavior using a neurobehavioral scale were measured every day. Subsequently the animals were anesthetized and perfused. Brains were cryopreserved and 20 µm cuts were obtained and cresyl violet staining. Cell number and dispersion were analyzed on hippocampal regions CA1 and CA3. Data for body weight, water and food intake were analyzed using a 2-way ANOVA and Duncan as post-hoc test, while neurobehavioral scale and histological data were analyzed using Kruskal-Wallis and Kolmogorov-Smirnov test as post-hoc. Statistical analysis showed significant differences by groups and days in body weight, water and food intake. In the neurobehavioral scale, G4, G5 and G6 were different between them and with the rest. In G4, impairment was observed in day 1 post-TBI and continued until day 7 post-TBI; in G5, the impairment lasted from day 1 to 5 post-TBI; while in group 6, the impairment only persists until day 3 post-TBI. In relation to histological data we didn't find differences in cell number, but we found statistical differences in percentage of dispersed cells in CA3; G4 showed more dispersion than G1, G2 and G3. All these data indicate a beneficial effect of rTMS on the rodent model of TBI used. Repetitive TMS could have possible therapeutic effect in head injury

Disclosures: L. Verdugo: None. **A. Hernandez-Chavez:** None. **E. Hernandez -Lopez:** None. **A. Garcia-Esponoza:** None. **F. Estrada-Rojo:** None. **M. Martinez-Vargas:** None. **L. Navarro:** None. **A. Perez-Arredondo:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.18/G35

Topic: C.10. Trauma

Support: Georgia Research Alliance

Title: water soluble analog of progesterone for the treatment of traumatic brain injury

Authors: *B. WALI[1], I. SAYEED[1], D. GUTHRIE[2], N. TURAN[3], D. LIOTTA[2], M. NATCHUS[2], D. G. STEIN[1];
[1]Emergency Med., [2]Chem., [3]Neurosurg., Emory Univ., Atlanta, GA

Abstract: BACKGROUND: Despite substantial body of experimental preclinical work documenting progesterone (PROG) as a neuroprotective agent, two recently completed phase III clinical trials show efficacy in the treatment of moderate to severe TBI. Although a number of problems with trial designs have been published, the use of PROG as a therapeutic agent also has practical limitations, including its poor solubility, stickiness in the intravenous bag and the instability of its formulation. The purpose of this study was to develop and optimize a PROG analog that is more potent, stable and easily administered by direct injection in field conditions. Here we present data on EPRX-1723 which has been tested in a well-established rat model of traumatic brain injury followed by functional/behavioral outcome measures to determine its efficacy. **Methods:** CCI was induced with a magnetic contusion device and rats were randomly assigned to different treatment groups. At 24 h, animals were killed and brains were removed for edema assay. For behavioral outcome dose of 10 mg/kg EPRX-1723 and PROG were given one hour post-injury and at 6, 24, hours and 2, 3, 4, 5, 6, and 7 days post-injury. The animals were tested pre-injury to establish a baseline of performance on grip strength and sensory neglect, then were retested at 4, 9 and 21 days post-TBI. MWM testing started on Day 11. A probe trial was given at 19d post injury. At 22d post injury, rats were perfused and brains extracted and processed for lesion size. **Results:** EPRX-1723 (10 mg/kg) when administered intramuscularly immediately after TBI, significantly reduced cerebral edema level, and improved recovery from motor, sensory and spatial learning deficits as well as or better than native PROG. Pharmacokinetic investigation of EPRX-1723 after a single intramuscular injection in rats revealed that the prodrug was rapidly converted to the active metabolite EPRX-036, which can be seen in plasma in a dose dependent manner thus demonstrating first order elimination kinetics. Similar measurements in brain tissue show that levels of EPRX-036 were present in concentrations that were slightly lower than those in plasma confirming that it readily crosses the blood brain barrier. **Conclusion:** Recent Phase III trials with PROG did not reveal benefit on a quality of life outcome measure_but the treatment delays ranged between at least 4-9h post-injury. Our results suggest that the water soluble EPRX-1723 represents a substantial advantage over those formulations used in the trials. EPRX-1723 may eventually provide a substantial advantage over PROG in that it can be given in the field by first responders and thereby reduce the time between injury and treatment.

Disclosures: **B. Wali:** None. **I. Sayeed:** None. **D. Guthrie:** None. **N. Turan:** None. **D. Liotta:** None. **M. Natchus:** None. **D.G. Stein:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.19/G36

Topic: C.10. Trauma

Support: VA Merit Award

Title: Modulation of autophagy by lanthionine ketimine improves outcome following traumatic brain injury

Authors: *M. E. HARRIS-WHITE[1], A. POTESHKINA[2], M. JOHNSON[2], P. ESLAMI[2], K. HENSLEY[3];
[1]UCLA & Veterans Administration-Greater Los Angeles, Los Angeles, CA; [2]Veterans Administration-GLA, Los Angeles, CA; [3]Univ. of Toledo, Toledo, OH

Abstract: Widespread traumatic damage to axons, termed diffuse axonal injury (DAI), may be a central contributor to functional/behavioral deficits following traumatic brain injury (TBI). Studies suggest axonal injury is not limited to time of injury and that progressive axonal injury continues many days post injury. Protein quality control and degradation play important roles in CNS homeostasis, particularly during injury or disease. The fidelity of the autophagic process is particularly relevant to CNS cells, and in particular, post-mitotic neurons that utilize autophagy to selectively target misfolded or aggregated proteins and defective organelles for removal. The role of autophagy in TBI is clouded by conflicting reports of autophagy being both detrimental and beneficial. Although there is substantial evidence that markers of autophagy are increased in both human and animal brains following TBI, it is not clear whether those changes result in functional autophagy. We have recently demonstrated that the axonal scaffolding protein, CRMP2, is involved in autophagy as engineered knockdown of CRMP2 reduces autophagy flux. Further, Lanthionine ketimine ethyl ester (LKE), binds to CRMP2 and stimulates autophagy in mammalian CNS cells. In this study, we utilized the mouse central fluid percussion model, a model of diffuse axonal injury. The progressive nature of DAI suggests that there is a period of time in which a pharmacological treatment might be effective to stabilize neuronal architecture, stimulate productive autophagy and allow repair mechanisms to function. In the present study we show that LKE, a bioavailable derivative of a natural brain sulfur amino acid metabolite, lanthionine ketimine, which we have previously shown to alleviate pathology and slow cognitive decline in the 3xTgAD mouse model, can spare cognition and pathology following DAI through mechanisms involving CRMP2 and autophagy modulation. To this effect, 30 minutes following a moderate TBI, and throughout the survival period, LKE was administered and mice subsequently evaluated for learning/memory impairments and biochemical and histological changes over a 5 week period.

Disclosures: **M.E. Harris-White:** None. **A. Poteshkina:** None. **M. Johnson:** None. **P. Eslami:** None. **K. Hensley:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xonovo.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.20/G37

Topic: C.10. Trauma

Support: ADDF

DePaul-RFUMS Pilot Award

Title: Stabilizing intracellular calcium channels prevents acute and sustained tau pathology in traumatic brain injury and AD models

Authors: N. KAPECKI[1], S. FISHER[1], B. DESAI[1], *C. A. BRIGGS[2], N. JAMNIA[3], D. PETERSON[1], D. KOZLOWSKI[3], G. STUTZMANN[1];
[1]Neurosci., Rosalind Franklin Univ. / The Chicago Med. Sch., North Chicago, IL; [2]Neurosci., Rosalind Franklin Univ., North Chicago, IL; [3]Biol. Sci., DePaul Univ., Chicago, IL

Abstract: The cellular mechanisms contributing to the proximal and long-term debilitating effects of a traumatic brain injury (TBI) are receiving increasing scrutiny. The tauopathies and amyloid deposits following a TBI are reminiscent of Alzheimer's disease (AD); indeed, TBI is a leading risk factor for sporadic AD. A common pathogenic feature between TBI and AD is calcium dysregulation through the ryanodine receptor (RyR). Acute dysregulated calcium signaling contributes to tau and amyloid aggregation within hours-to-days post-injury, while sustained calcium dysregulation likely contributes to the structural, synaptic, and behavioral deficits. In this study, we identify common features between TBI and AD to determine if similar upstream pathogenic mechanisms are recruited. If so, we hypothesize that our novel allosteric RyR modulators which ameliorate a multitude of AD features will be effective in reducing TBI-generated histopathology in the short-term (days), and prevent the progression to an AD phenotype in the long-term (weeks to months). We used a controlled-cortical impact TBI model in control and AD mice. Using immunohistochemistry against AD-associated tau and amyloid species in fixed brains sections, and *in vivo* 2-photon imaging in anesthetized animals, we show a rapid emergence of phospho-tau in the cortex and hippocampus of AD brains with a subsequent proliferation to the contralateral hippocampus within 7 days. Control mice showed a rapid phospho-tau response in the ipsilateral cortex and hippocampus, but this did not spread contralaterally during the 7-day time period. Amyloid plaques followed a similar pattern, with more aggressive aggregation and proliferation in the AD models. Upon treatment with a RyR-

targeted allosteric modulator within one hour of induced injury, and maintained for either 3 or 30 days post-injury, tau and plaque pathology were markedly reduced, as measured in fixed brain tissue and *in vivo*. Our data indicate that stabilizing RyR-evoked calcium signaling after a TBI impedes injury progression and reduces AD-related histopathology. We propose that by stabilizing aberrant intracellular calcium signaling soon after a TBI, we can generate both immediate and long-term therapeutic benefits and reduce the conversion to AD.

Disclosures: **N. Kapecki:** None. **S. Fisher:** None. **B. Desai:** None. **C.A. Briggs:** None. **N. Jamnia:** None. **D. Peterson:** None. **D. Kozlowski:** None. **G. Stutzmann:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.21/G38

Topic: C.10. Trauma

Title: Effect of creatine supplementation on cognition during hypoxia in mild traumatic brain injury

Authors: *C. E. TURNER, W. BYBLOW, S. BARKER-COLLO, R. KYDD, N. GANT; The Univ. of Auckland, Auckland, New Zealand

Abstract: The brain relies on an uninterrupted supply of oxygen in order to function optimally. Dysfunctional oxidative metabolism occurs with many neurological conditions, including mild traumatic brain injury (mTBI). Creatine is a compound that replenishes cellular energy without oxygen and can be administered as a dietary supplement. Creatine is capable of improving cognitive functions during oxygen deprivation [1] and may have similar effects for those recovering from mTBI. The aim of this study was to assess the effects of dietary creatine monohydrate supplementation on brain creatine and cognitive functions after mTBI. Spectroscopy was used to measure neural creatine availability. Neuropsychological assessments were conducted during a hypoxia protocol that induces cognitive impairments similar to those experienced after mTBI [2]. Neuropsychological function was measured at baseline and following 7 days of dietary creatine supplementation during the hypoxia intervention. Participants adhered to the 7 day supplementation regime, confirmed by an 8% increase in creatine within sensorimotor cortex. Creatine improved hypoxia-induced impairments in a range of cognitive domains scores that are commonly impaired following mild TBI. Verbal, visual and composite memory domains, psychomotor speed, and an overall neurocognitive index appear to be protected from oxygen deprivation by creatine supplementation. An enhanced energy-buffering capacity associated with augmented neural creatine stores likely increases energy provision in metabolically-vulnerable brain tissue. These preclinical findings suggest that creatine has utility to improve brain function in patients recovering from mTBI. References

[1]Turner CE, Byblow WD, Gant N. Creatine supplementation enhances corticomotor excitability and cognitive performance during oxygen deprivation. J Neurosci 2015; 35(4): p. 1773-1780. [2]Turner CE, Barker-Collo SL, Connell CJ, Gant N. Acute hypoxic gas breathing severely impairs cognition and task learning in humans. Physiol Behav 2015; 142: p. 104-110.

Disclosures: C.E. Turner: None. **W. Byblow:** None. **S. Barker-Collo:** None. **R. Kydd:** None. **N. Gant:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.22/G39

Topic: C.10. Trauma

Title: The efficacy of progesterone depends on the traumatic brain injury model

Authors: *A. M. CHOO[1], R. KOMLO[1], M. MANZANO[1], A. BARBOZA[1], Q. CHANG[1], T. HANANIA[2];
[1]PsychoGenics, Montvale, NJ; [2]PsychoGenics, Tarrytown, NY

Abstract: Progesterone was previously reported to improve outcomes in pre-clinical studies of traumatic brain injury. Recent phase 3 clinical trials, however, reported no clinical benefit of progesterone treatment for moderate-to-severe as well as severe traumatic brain injuries. Given the heterogeneity in human traumatic brain injuries, we aimed to reassess the effectiveness of progesterone treatment in two pre-clinical traumatic brain injury models. We compared progesterone treatment (16 mg/kg for 5 days) in mechanically identical controlled cortical impacts to the medial frontal cortex and the parasagittal cortex in rats. During the first week following injury, progesterone improved motor performance on the beam balance in both injury models. In the Morris water maze test, progesterone improved learning and memory only in animals that had received impacts to the medial frontal cortex and not in the parasagittal injury model. In the elevated plus maze, lesions to the medial frontal cortex increased the time the rats spent in the open arms which may indicate decreased anxiety and greater risk-taking behavior. This behavior was not attenuated by progesterone. The increase in the open arm time was modest in parasagittal injuries. Nonetheless, progesterone attenuated this modest increase back to levels similar to that of sham surgical controls. These data illustrate that the efficacy of post-traumatic progesterone treatment may depend on the brain region injured. Hence, the clinical translation of progesterone could benefit from additional stratification of patients accounting for the position of the brain lesions.

Disclosures: A.M. Choo: None. **R. Komlo:** None. **M. Manzano:** None. **A. Barboza:** None. **Q. Chang:** None. **T. Hanania:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.23/G40

Topic: C.10. Trauma

Support: the McKnight Brain Research Foundation, the Brain and Spinal Cord Injury Research Trust Fund

National Institutes of Health (NS046400)

Title: Hippocampal degeneration after traumatic brain injury: the roles of the PGE_2 EP1 receptor

Authors: A. V. GLUSHAKOV[1], J. M. GALVIS[1], S. L. SOLASKI[1], *S. DORÉ[2];
[1]Anesthesiol., [2]Dept. of Anesthesiology, Neurology, Psychiatry, Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Over the past decade, PGE_2 EP1 receptor blockers have been studied as a promising strategy for the treatment of neurological disorders and as a potential safer alternative to the cyclooxygenase-2 inhibitors. Preclinical data have demonstrated their efficacy in the treatment of ischemic and excitotoxic conditions by improving behavioral and anatomical outcomes and by promoting cell survival. However, according to recent reports, EP1 receptor roles are complex and the neuroprotective effects of its inhibition might be compensated or overpowered by adverse effects or toxicity in models of brain trauma and intracerebral hemorrhage. Consequently, the goal of this study was to investigate the effect of a selective EP1 receptor antagonist, SC-51089, on delayed neurodegeneration induced by traumatic brain injury (TBI) using a controlled cortical impact (CCI) model with two different injury magnitudes in mice. The data demonstrate that neurological deficit scores at 24 and 48 h after CCI rose with increasing injury magnitude. Repeated post-treatment with 20 ug/kg of SC-51089 has no significant effects on neurological deficit scores compared to vehicle groups with either magnitude. Of interest, 10 days after the severe CCI in the SC-51089 treatment group, the delayed hippocampal tissue loss was greater compared to controls. In mild-to-moderate TBI, the significant change in viable cell density was observed in the DG region of the contralateral hippocampi, whereas in its ipsilateral counterparts and other hippocampal regions, no statistically significant differences were observed. No significant changes in areas covering the pyramidal or granule cell layers in CA1-3 and DG regions, respectively, between corresponding control and SC-51089 treatment groups, were observed, suggesting that the changes in density are not due to hippocampal edema. The data, in combination with published reports, suggest that the EP1 inhibition worsened delayed degenerative processes in the hippocampus at sub-acute time points after TBI, and that this effect is more profound with increased trauma severity, likely due to the increased contribution of hemorrhagic injury.

Disclosures: **A.V. Glushakov:** None. **J.M. Galvis:** None. **S.L. Solaski:** None. **S. Doré:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.24/G41

Topic: C.10. Trauma

Support: Nutricia Research

Title: A specific multi-nutrient intervention, designed to enhance synapse formation and function, improves functional outcome following traumatic brain injury

Authors: *O. THAU ZUCHMAN[1], P. N. PALLIER[1], M. DAVIES[1], M. GROENENDIJK[2], M. C. DE WILDE[2], J. L. TREMOLEDA[1], A. T. MICHAEL-TITUS[1];
[1]Queen Mary Univ. of London, London, United Kingdom; [2]Nutricia Advanced Med. Nutr., Nutricia Res., Utrecht, Netherlands

Abstract: Traumatic brain injury (TBI) leads to major neurological impairment and at present there is no satisfactory treatment for this condition. Recent clinical trials in Alzheimer's disease have demonstrated the efficacy of Fortasyn® Connect (FC), a specific multinutrient combination that was designed to compensate for the loss of neuronal membranes and synapses in dementia patients. This specific multi-nutrient combination contains essential nutritional precursors and cofactors such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), choline, uridine monophosphate, phospholipids, folate, vitamins B6, B12, C, E and selenium, which synergize to support neuronal membrane formation and function. Recent clinical trials have shown improvement in cognitive function and increased cortical connectivity in Alzheimer's disease patients following daily intake of this multi-nutrient combination. TBI can lead to major tissue disruption, neuroinflammation and axonal damage. In the long-term TBI increases the risk of developing neurodegenerative conditions. Because of the relevance of neuronal membranes and synapses also in TBI, we hypothesised that a diet supplemented with this specific multi-nutrient combination could have protective and pro-regenerative effects after TBI. We investigated in a mouse model of TBI whether this supplemented diet can counter the tissue injury occurring after TBI and lead to an improved neurological outcome. Adult male C57/BL6 mice received an injury by controlled cortical impact (CCI) and were then assessed on a multitude of behavioural tasks, carried out at various time intervals, for 70 days post-injury (dpi). Following TBI, animals were fed daily with a control diet or the FC until the end of the study. FC significantly attenuated over the whole duration of the study the global deficit post-TBI assessed using a modified neurological severity score (mNSS). FC also decreased the injury-induced impairment in the Rotarod test (used to explore motor coordination, balance and strength), which was carried out in the injured animals in the first 3 dpi. Following CCI, mice

developed a marked impairment in spatial memory, which was assessed in the Morris Water Maze (MWM) between day 13 and day 18 post-TBI. The dramatic injury-induced deficit revealed in the probe trial in the MWM, was corrected by FC diet. These results suggest that a diet supplemented with FC multi-nutrient combination, which has already been shown to be very well-tolerated in patients with dementia, has marked therapeutic potential in TBI.

Disclosures: O. Thau Zuchman: None. **P.N. Pallier:** None. **M. Davies:** None. **M. Groenendijk:** A. Employment/Salary (full or part-time):; Nutricia Research. **M.C. de Wilde:** A. Employment/Salary (full or part-time):; Nutricia Research. **J.L. Tremoleda:** None. **A.T. Michael-Titus:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.25/G42

Topic: C.10. Trauma

Support: NIH Grant NS-081370 (AJR)

Office of the Dean, Univ. of Tennessee Health Sci. Ctr. (AJR)

Neuroscience Institute at Univ. of Tennessee Health Sci. Ctr. (AJR)

College of Pharmacy, Univ. of Tennessee Health Sci. Ctr. (BMM)

Methodist Hospitals Endowed Professorship in Neuroscience (AJR)

Title: Neurons are lost in brain regions controlling movement and fear after mild TBI and rescued by a CB2 receptor inverse agonist

Authors: W. BU[1], H. REN[1], Y. DENG[1], N. DEL MAR[1], N. M. GULEY[1], Y. GAO[1], M. G. HONIG[1], S. A. HELDT[1], B. M. MOORE, II[2], *A. J. REINER[1];
[1]Anat. & Neurobio., [2]Pharmaceut. Sci., The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: In our closed-head model of mild TBI (mTBI), a high-pressure air blast is targeted to the left side of the mouse cranium, producing axonal injury, microglial activation, and motor and emotional deficits, but no overt contusive injury (Heldt et al. Frontiers Neurol. 2014). Here we report neuronal loss in several specific brain regions by 2-3 months after blast. Using stereology, we found 20% fewer NeuN-immunolabeled neurons in cortex on both sides, and 30% fewer NeuN-immunolabeled neurons in striatum on both sides in mice that had received 50-60 psi blasts as compared to their sham blasted littermates. For substantia nigra, 33% of tyrosine hydroxylase-immunostained dopamine neurons were lost on the left side and 13% on the right. Since our previous work showed that treatment with the novel cannabinoid type-2 receptor

(CB2) inverse agonist SMM-189 alleviates the motor deficits caused by mTBI in our model, by converting brain microglia from a pro-inflammatory M1 phenotype to a pro-healing M2 phenotype (Reiner et al., Int J Mol Sci. 2014), we examined the effects of SMM-189 on neuronal loss. We found that daily SMM-189 intraperitoneal injection, for two weeks beginning just after blast, yielded about 66% bilateral rescue of the cortical neuron loss, about 50% bilateral rescue of the striatal neuron loss, and near complete rescue of the dopaminergic nigral neuron loss. This, together with the rescue of corticospinal tract axons (Reiner et al., Int J Mol Sci. 2014), may contribute to the post-mTBI motor improvement seen with SMM-189 treatment. Mice subjected to blast mTBI also exhibit increased fearfulness that is alleviated by SMM-189. Given the role of basolateral amygdala (BLA) in fear control, we examined specific neuronal populations within it. Blast produced a 33% loss of thy1+ pyramidal neurons, no significant loss of thy1-negative BLA neurons, and a 47% loss of parvalbuminergic (PARV+) inhibitory BLA interneurons. The preferential loss of thy1+ neurons (which suppress fear) compared to thy1-negative neurons (which predominantly are pyramidal neurons that promote fear) may explain how mTBI produces increased fearfulness. Furthermore, the substantial loss of PARV+ interneurons associated with increased fear after mTBI suggests that the PARV+ interneurons may preferentially inhibit the thy1-negative fear-promoting pyramidal neurons. SMM-189 treatment rescued about 33% of the thy1+ neuron loss and about half of the PARV+ neuron loss. Thus, fear deficits after mTBI may result from loss of fear-suppressing neuron types in BLA, and SMM-189 may produce its benefit by their rescue.

Disclosures: W. Bu: None. **H. Ren:** None. **Y. Deng:** None. **N. Del Mar:** None. **N.M. Guley:** None. **Y. Gao:** None. **M.G. Honig:** None. **S.A. Heldt:** None. **B.M. Moore:** None. **A.J. Reiner:** None.

Poster

309. Traumatic Brain Injury: Therapeutic Strategies II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 309.26/G43

Topic: C.10. Trauma

Support: VA IK2RX001479

VA IRX001097A

Title: Hippocampal neurophysiology in awake behaving swine after diffuse brain injury

Authors: *P. KOCH[1], A. TEKRIWAL[1], M. GROVOLA[2], A. V. ULYANOVA[1], D. K. CULLEN[2,1], J. A. WOLF[2,1];
[1]Dept. of Neurosurgery, Ctr. for Brain Injury and Repair, Univ. of Pennsylvania, Philadelphia, PA; [2]Neurosurg., Philadelphia VA Med. Ctr., Philadelphia, PA

Abstract: We have previously established an acute recording methodology to interrogate hippocampal circuitry after diffuse brain injury (DBI) in a swine model of rotational injury. Injuries were administered over a range of coronal rotational accelerations (180-260 rad/sec) that induced little or no loss of consciousness (< 15 min), yet exhibited axonal pathology. Limitations of electrophysiological recording under anesthesia have led us to develop a chronic hippocampal electrode implantation model in the awake, freely moving swine, allowing examination of hippocampal networks engaged in relevant behavior after injury. Repeated concurrent electrophysiological and behavioral measures enable examination of how network level interactions may be disrupted after DBI. We have developed a stereotaxic surgical technique for precise implantation of a custom 32-channel silicone electrode into the swine hippocampus that allows for recordings of both single units in layer CA1 and dentate, as well as simultaneous laminar field potentials while the animal is awake and freely moving during behavioral tasks. We have also developed a novel object recognition task for swine, a behavior known to be hippocampal dependent. Pigs were trained on this task prior to electrode implantation. Preliminary behavioral results indicate that sham injured swine reliably interact longer with novel objects versus familiar objects. Moreover, we demonstrate robust extracellular field potentials out to 6 months post-implantation, as well as stable unit recordings pre- and post-implantation. Using spectral density analysis we report a prominent peak in hippocampal theta rhythm power in the freely behaving pig with positive shifts in peak frequency and peak power during periods of locomotion. This dominant hippocampal rhythm has previously been shown to be disrupted in rodent traumatic brain injury models. Here we demonstrate the feasibility of combining chronic hippocampal electrophysiological recordings with concurrent behavior in freely moving large animals. Combining this methodology with our established DBI model in pigs may reveal mechanisms of trauma-induced network dysfunction which may lead to innovative neuromodulatory therapies.

Disclosures: **P. Koch:** None. **A. Tekriwal:** None. **M. Grovola:** None. **A.V. Ulyanova:** None. **D.K. Cullen:** None. **J.A. Wolf:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.01/G44

Topic: C.10. Trauma

Title: Precision and reliability of diffusion-weighted magnetic resonance imaging in healthy muscles of the lower extremity

Authors: *J. G. MCPHERSON[1,2], M. WASIELEWSKI[2], J. M. ELLIOTT[2];
[1]Biomed. Engin., Florida Intl. Univ., Miami, FL; [2]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL

Abstract: Diffusion-weighted magnetic resonance imaging (DW-MRI) is commonly used to identify regions of injury within the central nervous system (CNS). However, in cases when CNS injury is suspected but not visible - for example a mild, diffuse contusion of the spinal cord - DW-MRI of skeletal muscle may provide an alternative approach to assessing the likelihood of neural damage. Indeed, animal models have demonstrated an increased apparent diffusion coefficient in denervated skeletal muscle compared to typically innervated muscles. Further, these changes appear to precede findings from electrophysiological testing. Particularly in cases of suspected spinal cord injury arising from a motor vehicle collision, rapid and early changes in muscle properties distal to the site of trauma may be strong predictors of the rate and extent of recovery. Thus, the adaptation of DW-MRI to skeletal muscle holds considerable promise from both diagnostic and prognostic standpoints. Despite this, the precision and reliability of DW-MRI in skeletal muscle has yet to be established in individuals without neurological injury, significantly limiting its utility in cases of suspected CNS damage. Therefore, the objective of this pilot study was to investigate the precision, intra-rater, inter-rater, and short and long-term reliability of DW-MRI in muscles of the lower leg. Six neurologically uninjured individuals (3 male, 3 female) provided informed consistent and underwent a DW-MRI scan of the bilateral plantar and dorsiflexor muscles. Three individuals returned approximately 6 months later for a duplicate scan of the same muscle groups. DW-MRI scans were performed on a Siemens Trio 3T MRI scanner. Diffusion gradients were applied in 3 orthogonal directions at b-values of 0, 100, 300, and 500 s/mm2. Two region of interest (ROI) definitions were used: the first encompassing the entire area of the muscles at a given level and the second defined as the largest circular region that could be fully contained within the border of the muscles. Novice raters created and determined all ROI. Precision statistics included root-mean-square coefficient of variation (RMS-CV) and least significant change (LSC). Intra- and inter-rater reliabilities were assessed using intra-class correlation coefficients. RMS-CV values averaged 2.95 across raters and muscles, with LSC values averaging 8.18%. Intra- and inter-rater agreement averaged 98% for all comparisons and ROI definitions, with excellent short and long-term reliability. These results serve as essential validation of DW-MRI in skeletal muscle and will enable successful interpretation of DW-MRI results in future investigations.

Disclosures: J.G. McPherson: None. **M. Wasielewski:** None. **J.M. Elliott:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.02/H1

Topic: C.10. Trauma

Title: Assessment of thoracic and cervical spinal cord injuries using intercostal motor evoked potentials in humans

Authors: *F. D. BENAVIDES[1], A. J. SANTAMARIA[1], A. Y. FLORES[2], J. D. GUEST[1];
[1]The Miami Project to Cure Paralysis, Univ. of Miami, Miller Sch. of Med., Miami, FL;
[2]Pediatrics - Critical Care 2, Univ. of Miami, Miami, FL

Abstract: Background. Trans-cranial motor evoked potentials (tcMEPs) are a quantitative tool to assess conductivity of motor pathways, typically to limb muscles. However, tc stimulation can also be used to assess conduction in individual segments of the thoracic spinal cord by recording MEPs from intercostal muscles (ICs). The possibility to establish a functional motor level in the disrupted thoracic spinal cord could allow tracking of changes in adjacent segments and serve as a tool to evaluate natural recovery and/or the impact of therapeutics. In 1965, Guttman and Silver published observations that intercostal muscles were triggered reflexively in complete quadriplegics by breathing. However, we noted during the tcMEP study of a T2 thoracic AIS A subject that IC were prominent well below the sensory level and subsequently sought to understand this unexpected finding. Objectives. To characterize and analyze intercostal MEPs in normal and injured subjects after thoracic spinal cord injury. Methods. MEPs were recorded in 4 paraplegic patients (T2-T6, ASIA-A) utilizing surface electrodes located in the IC spaces, placed to avoid overlying surface muscles; recordings from subjects were compared to recordings collected in 4 control volunteers. The SCI subjects were enrolled in an FDA and IRB approved clinical trial at the University of Miami. Results. MEP amplitudes were decreased in segments below the injury site in SCI subjects, latencies were found slightly delayed or unchanged. In all subjects amplitudes and latencies were consistent within samples collected during the same session; latencies were more consistent than amplitudes between follow-up sessions and subjects. tcMEP evoked shorter and higher amplitude MEPs when the coil was placed over the vertex (compared to lateral cortical position). Conclusion. MEPs can be recorded with surface or needle electrodes from the intercostal spaces. Amplitudes change is the most reliable marker of injury level. Evoked signal is found in segments below complete injuries, suggesting a multi segmental innervation in IC muscles. ICs-MEPs may be used to help in establishing a functional level in thoracic injuries as well as to assess the safety and progression of cell transplantation strategies.

Disclosures: F.D. Benavides: None. **A.J. Santamaria:** None. **A.Y. Flores:** None. **J.D. Guest:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.03/H2

Topic: C.10. Trauma

Title: Use of swimming test for evaluating spasticity after contusive spinal cord injury

Authors: ***Y. RYU**[1,2], N. FUJITA[1], T. OGATA[2];
[1]Grd. Sch. of Agr. and Life Sci., Univ. of Tokyo, Tokyo, Japan; [2]Dept. of Rehabil. for Motor Dysfunction, Natl. Rehabil. Ctr., Saitama, Japan

Abstract: Spasticity is a common disabling complication after spinal cord injury (SCI) which includes multiple symptoms such as muscle spasm, clonus and hyperreflexia. However, these symptoms of spasticity have not been fully recapitulated in animal SCI models. Also, methods for monitoring the symptoms of spasticity repeatedly after SCI have not been established yet. By these limited conditions of spasticity models, not only the mechanism but also the assessment of experimental intervention against spasticity remains unclear. Here, we attempted to quantitatively analyze spasticity including muscle spasm and clonus after SCI by swimming test and confirm the feasibility of the test by cage observation with simultaneous electromyography (EMG) recording from hind limb of rats. Rats were given 250kd contusive injury with Infinite Horizon impactor at Th8 level. Three weeks after SCI, EMG wires were inserted into tibialis anterior muscle and gastrocnemius muscle. The SCI rats weekly perform swimming test starting at 3 weeks after SCI. In each session, rats swam for 100 cm distance for ten times. The spastic behaviors during swimming were confirmed by simultaneously recorded EMG pattern. On the other hand, cage observation during 20 hour was done at 4 weeks after SCI. The spastic EMG activities were identified and confirmed by video monitoring. The number of spastic episode during 20 hour was recorded. Also, Hoffman(H) reflex test was taken at 6 weeks after SCI. According to the frequency of the spastic symptoms during swimming test, SCI rats were categorized into spasticity positive group or spasticity negative group. There were no statistical differences in the histological lesion size at epicenter between two groups. About 70% of rats with SCI showed spastic behavior classified by swimming test. The prevalence of spastic behavior of spasticity positive group during ten times swimming (one session) constant from 4 to 6 weeks after injury. Spasticity positive groups showed more spasm and clonus during cage observation and more hyperexcitablility tested by rate-dependent depression of the H reflex test than spasticitiy negative group. The EMG patterns during swimming test confirm that the episodic behaviors are related to spasticity. In addition, the consistency between swimming test and cage observation indicates the feasibility of swimming test to represent the spasticity of the rat. Based on our finding, we assume that swimming test can be used for evaluating spasticity with good reproducibility. For future work, we are now examined what factors related to present spasticity at tissue and molecular level.

Disclosures: **Y. Ryu:** None. **N. Fujita:** None. **T. Ogata:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.04/H3

Topic: C.10. Trauma

Support: MD-PhD scholarship of the Swiss Academy of Medical Sciences (SAMS)

the Swiss Continence Foundation

advanced ERC grant (Nogorise) to MES

the Christopher and Dana Reeve Foundation

Swiss National Science Foundation

Title: Long-term longitudinal urodynamic findings in awake spinal cord injured rats

Authors: *M. P. SCHNEIDER[1], A. K. ENGMANN[1], A. JESKE[1], M. E. SCHWAB[1], T. M. KESSLER[2];
[1]Univ. of Zürich, Zürich, Switzerland; [2]Neuro-Urology, Univ. of Zürich, Balgrist Univ. Hosp., Zürich, Switzerland

Abstract: We recently developed a novel urodynamic model allowing repetitive measurements of both bladder and external urethral sphincter function at different time points in the same rat under fully awake conditions (Schneider MP et al., BJU International 2015). We now applied this model to assess the development of lower urinary tract dysfunction as a consequence of spinal cord injury (SCI) in adult rats over 6 weeks. A urodynamic catheter into the bladder and external urethral sphincter electromyography (EMG) electrodes were implanted into female Lewis rats. Two weeks after implantation, baseline urodynamic investigation showed a normal fast, large volume voiding response to bladder filling. The external urethral sphincter EMG showed peak activity at the high filling state immediately before as well as after voiding and a lower, slow wave bursting activity during voiding and tonic activity in the non-voiding phase. We used 3 different types of spinal cord lesions at T8 level: complete microsurgical transection, or severe compression by a vascular clip leading to complete (180 sec. clip time) or large but incomplete (60 sec. clip time) lesions. Urodynamic measurements were performed weekly in awake animals upon 6 weeks after SCI. Histological investigation of the spinal cord was performed to assess lesion completeness after perfusion. In the control animals (n=10) without SCI, urodynamic and EMG findings were similar throughout the whole study duration and no pathological effects were observed. In contrast, almost all rats with SCI (n=15) developed detrusor sphincter dyssynergia (DSD) characterized by high tonic sphincter activity during the voiding phase, resulting in dripping and interrupted release and very prolonged voiding phases. Typically, like in human spinal cord injured patients, DSD developed slowly over time, about three weeks after injury in the rats. All these pathophysiological changes were highly reproducible and resemble closely the DSD state typically found in humans with SCI.

Disclosures: M.P. Schneider: None. **A.K. Engmann:** None. **A. Jeske:** None. **M.E. Schwab:** None. **T.M. Kessler:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.05/H4

Topic: C.10. Trauma

Support: US Dept of Defense Grant: W81XWH-11-2

Title: Biological effects of vibration simulating helicopter and MRAP transport on spinal cord injured pigs

Authors: *N. MANOUCHEHRI[1], F. STREIJGER[1], J. H. T. LEE[1], A. D. MELNYK[1,2], J. D. CHAK[1,2], S. TIGCHELAAR[1], K. SO[1], E. B. OKON[1], S. JIANG[1], R. KINSLER[3], K. BARANZANJI[3], P. A. CRIPTON[1,2], B. K. KWON[1,4];
[1]ICORD, [2]Departments of Mechanical Engin. and Orthopaedics, UBC, Vancouver, BC, Canada; [3]US Army Aeromedical Res. Lab., Fort Rucker, AL; [4]Dept. of Orthopaedics, Vancouver Spine Surgery Institute, UBC, Vancouver, BC, Canada

Abstract: Incidents that cause spinal cord injury (SCI) typically require vehicular transport via ambulance or helicopter to evacuate the patient to an emergency medical center. During ground or air transportation, the spinal cord injured patient is inevitably subjected to various degrees of vibration. Others have shown that exposure to a wide range of vibrations may contribute to the development of pathological changes in the spinal cord and can lead to acute and sub-acute alterations in the cellular environment. Within the setting of a compromised spinal cord, the effects of vibration from different modes of transportation on further injury have not been directly studied. This has particular relevance in military conflicts where helicopters and Mine-Resistant Ambush Protected (MRAP) vehicles are often used to transport soldiers who have suffered a SCI. The purpose of this study was to determine if the application of vibration relevant to these forms of transportation influence the injured spinal cord and subsequent recovery from it. We employed our porcine model of thoracic SCI in which the spinal cord was injured at T10 with a combination of weight-drop contusion and sustained compression. Shortly after injury, animals were either exposed to i) no vibration, ii) 3 hours of vibration simulating a military helicopter, or iii) 3 hours of vibration simulating MRAP ground ambulance transportation. The experimental setting allowed us to apply vibratory stimuli with controlled frequency, amplitude, and duration - these parameters were provided by the US military. CSF samples, taken at various intervals during and after vibration, were biochemically analyzed for the presence of inflammatory mediators and structural biomarkers. Hind-limb recovery was assessed by a locomotor rating scale, i.e. Porcine Thoracic Injury Behavior Scale (PTIBS) for 12 weeks after SCI. The spinal cord was evaluated histologically to quantify the extent of white and grey matter sparing through the injury site. We observed that the concentrations of various biomarkers such as GFAP, MCP-1, IL-6, and IL-8 were elevated in all groups between 6-12 hours post injury but

were very similar between the vibrated and non-vibrated groups. Hind-limb function was also very comparable between the three groups of animals throughout the 12 week recovery. As predicted from the biochemical and behaviour data, there were no histological differences in the amounts of spared white or grey tissue matter. Thus, in summary, this study suggests that exposure to whole-body vibration simulating helicopter or MRAP transportation does not result in significant behavioural or histological worsening after traumatic SCI.

Disclosures: **N. Manouchehri:** None. **F. Streijger:** None. **J.H.T. Lee:** None. **A.D. Melnyk:** None. **J.D. Chak:** None. **S. Tigchelaar:** None. **K. So:** None. **E.B. Okon:** None. **S. Jiang:** None. **R. Kinsler:** None. **K. Baranzanji:** None. **P.A. Cripton:** None. **B.K. Kwon:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.06/H5

Topic: C.10. Trauma

Support: NIH NS050243,

NIH NS059622,

NIH NS073636,

DOD CDMRP W81XWH-12-1-0562,

DVA 1I01BX002356-01A1

Craig H Neilsen Foundation # 296749

Indiana Spinal Cord and Brain Injury Research Foundation.

Title: Generating graded spinal cord contusion injury using Louisville injury system apparatus (LISA) in adult mice

Authors: ***X. WU**[1,2,3], W. QU[1,2,3], C. M. E. FRY[1,2,3], H. DAI[1,2,3], Y. ZHANG[4], C. SHIELDS[4], X.-M. XU[1,2,3];
[1]Spinal Cord and Brain Injury Res. Group, Indianapolis, IN; [2]Stark Neurosciences Res. Inst., Indianapolis, IN; [3]Dept. of Neurolog. Surgery, and Goodman Campbell Brain and Spine,, Indiana Univ. Sch. of Med., Indianapolis, IN; [4]Norton Healthcare, Norton Neurosci. Inst., Louisville, KY

Abstract: The Louisville Injury System Apparatus (LISA) impactor has been shown to be consistent, reliable, and reproducible in producing rat contusive spinal cord injury (SCI) due to its precise determination of the “0” point of spinal dorsal surface with a laser sensor and precise

generation of cord displacement (Zhang et al., J. Neurotrauma 25:1227-1240, 2008). However, the LISA device has yet to be tested in mice. In the present study, we examined whether the LISA impactor could be extended to creating graded contusive SCIs in mice. Three groups of C57Bl/6 mice received a T10 laminectomy followed by the creation of 0.2, 0.5 and 0.8mm spinal cord displacement injuries from the dorsal surface of the spinal cord using LISA. The sham group received laminectomy alone. Locomotor assessments using Basso Mouse Scale (BMS), grid walking, and TreadScan analysis were performed for up to 6 weeks post-injury. All mice were sacrificed at 7 weeks and the spinal cords containing the lesion epicenter were collected, sectioned, and examined. Our results showed that LISA produced accurate cord displacements in all 3 injury groups: 0.201 ± 0.0026 (mean ± standard deviation), 0.500 ± 0.0014, and 0.801 ± 0.0024, representing mild, moderate and severe injuries to the cord. The BMS and grid walking assessments showed statistically significant differences among the 3 injury groups. The TreadScan analysis allowed assessments of mice with a BMS score >5. For this criteria, the 0.2 and 0.5mm injury groups were assessed and significant differences in TreadScan measures were found between the 2 groups. Significant differences in TreadScan measures were also found between the 0.2mm and sham groups. Histological analysis showed statistically significant difference in lesion area, lesion length, and spared white matter at the injury epicenter among the 3 injured groups. We conclude that the LISA device can produce precise graded contusive SCIs in mice that result in severity-dependent behavioral and histopathological deficits.

Disclosures: **X. Wu:** None. **W. Qu:** None. **C.M.E. Fry:** None. **H. Dai:** None. **Y. Zhang:** None. **C. Shields:** None. **X. Xu:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.07/H6

Topic: C.10. Trauma

Support: US Dept of Defense W81XWH-14-1

Paralyzed Veterans of America #2962

Title: Spinal Cord pressure, blood flow and oxygenation and the effect of Duraplasty after SCI

Authors: ***K. SHORTT**[1], F. STREIJGER[1], N. MANOUCHEHRI[1], K. SO[1], E. OKON[1], B. K. KWON[1,2];
[1]ICORD (International Collaboration on Repair Discoveries), [2]Vancouver Spine Surgery Institute, Dept. of Orthopaedics, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Severe swelling of the human spinal cord is typically observed after traumatic spinal cord injury (SCI). Intrinsic cord swelling within the pia mater may result in increased

intraparenchymal pressure and reduced perfusion. The swollen cord can fill the subarachnoid space and be pushed up against the dura mater, causing a further increase in intraparenchymal pressure. In keeping with this, clinical studies of severe TBI have revealed that dural opening is required to relieve cerebral and improve blood flow and oxygen supply. Expansion duraplasty in human SCI is not routinely performed, but recently has been reported to reduce intraspinal pressure as measured by a subdural pressure probe (Phang et al, J. Neurotrauma, 2015). In this study, we directly measured intraparenchymal pressure, blood flow, oxygenation, and metabolic responses in our porcine model of SCI to evaluate these changes over the first week post-injury and to determine the intraparenchymal effects of expansion duraplasty. Our porcine model utilizes 20-25 kg Yucatan minipigs that are more similar to humans in their spinal cord anatomy and size than rodents, and importantly, have a notable CSF space around the spinal cord. This allows us to model the swelling and filling of the intrathecal space that is observed after human SCI. Following contusion plus compression SCI, animals were randomized to either having an intact dura or having a duraplasty to expand the subarachnoid space. Adjacent to the injury site we inserted monitoring probes directly into the parenchyma to measure spinal cord blood flow, oxygenation, metabolic responses (with microdialysis), and hydrostatic pressure. Monitoring was continued for 7 days. Our preliminary data reveals that adjacent to the injury site, the pressure within the spinal cord spiked at the time of impact and remained increased during the compression period. When the spinal cord was decompressed, a sharp pressure drop occurred, after which the pressure climbed gradually. The magnitude of pressure change away from the injury site was far less pronounced. Currently we are also compiling the data from the intraparenchymal blood flow, oxygenation, and microdialysis probes. In summary, we observed an increase in cord pressure and swelling following SCI, which is also routinely observed in individuals with acute SCI. Such cord swelling is poorly understood, and is quite likely to have an impact on neurologic recovery. Our data will provide fundamental insights into this phenomenon that could guide best practices and optimize neurologic outcome. Ref: Phang et al, J Neurotrauma. 2015 May 4. [Epub ahead of print] PubMed PMID: 25705999.

Disclosures: **K. Shortt:** None. **F. Streijger:** None. **N. Manouchehri:** None. **K. So:** None. **E. Okon:** None. **B.K. Kwon:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.08/H7

Topic: C.10. Trauma

Support: US Dept of Defense Grant: W81XWH-14-2

Wing for Life Grant: WFL-CA-013/14

Title: Hemodynamic management of acute spinal cord injury: Use and effects of common vasopressors on spinal cord blood flow, oxygenation and downstream metabolic responses after SCI in a porcine model

Authors: *K. SO[1], F. STREIJGER[1], N. MANOUCHEHRI[1], E. B. OKON[1], K. SHORTT[1], J. H. T. LEE[1], B. K. KWON[1,2];
[1]ICORD (International Collaboration On Repair Discoveries), [2]Vancouver Spine Surgery Institute, Dept. of Orthopaedics, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Early intervention strategies after a traumatic spinal cord injury (SCI) remain limited, with vasopressor support of mean arterial pressure and spinal cord perfusion being one of the few treatment options available. After an acute SCI, a drop in the Mean Arterial Pressure (MAP) is commonly observed, leading to a decrease in spinal cord blood flow (SCBF). Reduced cord perfusion and ischemia are major contributors to secondary damage. Thus, clinicians aim to restore adequate SCBF by augmenting the MAP with vasopressor drugs. However, such drugs have differing pharmacological properties and as such, each has its own distinct effect on restoring SCBF and minimizing ischemia. Therefore, a clinically relevant question is, which vasopressor agent is best suited to augmenting spinal cord perfusion and minimizing ischemia within the injured spinal cord? To answer this question, we compared three commonly used vasopressors, norepinephrine (NE), phenylephrine (PE), and dopamine (DA), to determine their intraparenchymal effects on SCBF, oxygenation and downstream metabolic responses after SCI. Using our pig model of a contusion/compression SCI, we compared the effects of NE, PE, and DA, during sustained compression and also after decompression. To mimic the clinical resuscitation goals of hemodynamic management for acute SCI patients, the MAP of experimental animals was increased by 20 mmHg. Throughout the experiment, the intraparenchymal oxygenation/blood flow response as well as metabolite levels of glucose, lactate, pyruvate, L/P ratio, glutamate and glycerol were assessed, both proximal and distal to the injury site. Preliminary data suggest that NE and PE both increase the blood flow and oxygenation proximal to the injury site. While PE does not increase blood flow as readily as NE close to the injury site, the oxygenation increases are similar. Increases in blood flow and oxygenation with DA treatment was only achieved at extremely high administration levels, far beyond what would be clinically relevant. Evaluation of metabolic responses using intraparenchymal microdialysis shows some increase in tissue glucose levels with Norepinephrine. Further study will clarify the differences in intraparenchymal responses to these vasopressor agents.

Disclosures: K. So: None. **F. Streijger:** None. **N. Manouchehri:** None. **E.B. Okon:** None. **K. Shortt:** None. **J.H.T. Lee:** None. **B.K. Kwon:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.09/H8

Topic: C.10. Trauma

Support: CIHR Grant: MOP-133513

US Dept of Defense Grant: W81XWH-14-1

Title: Changes in spinal cord metabolism, oxygenation and blood flow after acute SCI using a porcine model

Authors: *E. B. OKON[1], F. STREIJGER[1], N. MANOUCHEHRI[1], K. SHORTT[1], K. SO[1], B. K. KWON[1,2];
[1]ICORD (International Collaboration On Repair Discoveries), [2]Vancouver Spine Surgery Institute, Dept. of Orthopaedics, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Hypoxia and ischemia have been long recognized as common and potentially harmful events after spinal cord injury (SCI). This has led to the development of treatment strategies aimed at optimizing intraparachymal spinal cord blood flow (SCBF), which has been extensively studied after acute SCI. However, the relationship between SCBF, oxygenation, and downstream tissue ischemia has not been well established. In this study we evaluated the intraparenchymal blood flow, oxygenation and metabolic responses within the spinal cord during the (sub)-acute phases of SCI. Here, we used a porcine model of SCI involving a combination of contusion and compression components. Using intraparenchymal microdialysis, extracellular fluid from the spinal cord at 1.2 and 3.2 cm caudal to the center of the impact was interrogated for up to 7-days. These samples were analyzed for various markers of cellular damage, ischemia and energy status, including lactate, pyruvate, L/P ratio and glucose. Furthermore, additional sensors were implanted into the cord at the same position to measure responses of oxygenation and blood flow. Proximal to the injury site (1.2-cm), SCBF and oxygenation decreased dramatically following SCI and while the cord remained compressed. Following decompression, they recovered slightly but remained below baseline. At around 2 days post-injury, SCBF levels steadily increased such that at the end of the experiment, levels reached far above baseline. Oxygenation levels tended to increase after day 1. Glucose values decreased significantly upon SCI, and subsequently returned to baseline by day 1. Lactate to pyruvate (L/P) ratio, a marker for tissue ischemia, increased significantly within minutes after SCI and cord compression and subsequently decreased after decompression. However, the L/P ratio increased again to levels 5-fold above baseline by day 7. Distal from the injury site (3.2-cm), the response to SCI, compression and decompression was minimal, however, over the course of 7-days, gradual changes were observed. Glucose levels began to fall below baseline around 5 days post-injury. In addition, oxygenation and SCBF decreased slowly but continuously over time, while the L/P ratio steadily increased up to 4-fold above baseline. Taken together, our preliminary data sheds light on the dynamic changes that occur with oxygenation, SCBF, and metabolic responses in the penumbra of the traumatic spinal cord injury site.

Disclosures: **E.B. Okon:** None. **F. Streijger:** None. **N. Manouchehri:** None. **K. Shortt:** None. **K. So:** None. **B.K. Kwon:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.10/H9

Topic: C.10. Trauma

Title: Effect of injury severity on serum and cerebrospinal fluid miRNA expression profile after traumatic spinal cord injury in pigs

Authors: *S. S. TIGCHELAAR[1], F. STREIJGER[2], S. SINHA[3], N. MANOUCHEHRI[2], K. SO[2], I. MALENCIA[4], A. COURTRIGHT[4], T. BEECROFT[4], K. VAN KEUREN-JENSEN[4], C. NISLOW[3], B. KWON[2,5];
[1]ICORD, Vancouver, BC, Canada; [2]ICORD, UBC, Vancouver, BC, Canada; [3]Dept. of Pharmaceut. Sciences, UBC, Vancouver, BC, Canada; [4]TGen, Phoenix, AZ; [5]Orthopaedics, Vancouver Spine Surgery Institute, UBC, Vancouver, BC, Canada

Abstract: With limited treatment options currently available to clinicians, there is an urgent need for non-invasive biomarkers to aid in the scientific development and clinical validation of novel therapies for acute spinal cord injury (SCI). Micro RNAs (miRNAs) are small regulatory noncoding RNA molecules approximately 22 nucleotides in length that mediate post-transcriptional silencing of gene expression via the interaction with specific sequences in target messenger RNA. The current body of literature suggests that miRNAs influence a wide range of biological processes such as proliferation, differentiation, adult neurogenesis, synaptic plasticity and apoptosis. Many miRNAs are highly expressed in the adult nervous system in a temporally and spatially controlled manner in normal physiology, and are also directly implicated in the pathogenesis of various neurodegenerative diseases including traumatic SCI. Besides their specific spatial, temporal and cellular-level expression, our interest in microRNAs furthermore stems from their stability within blood, making it possible for blood samples to be utilized in order to measure markers specific to the injured central nervous system. In this study, we compared the miRNA expression profiles of serum and cerebral spinal fluid (CSF) between various injury severities in a porcine model of SCI. Female Yucatan minipigs underwent a T10 SCI using a weight drop impactor followed by compression for 5 minutes. Animals were grouped into three levels of injury severity, which were induced by altering the height of the weight drop (10, 20, and 40 cm). Next-generation sequencing technology was used to compare effects of injury severity on miRNA levels obtained daily over a period of 7 days post-injury. Extracellular miRNAs were then isolated and sequenced using the Illumina HiSeq system and the generated data was aligned using miRDeep2 and tested for differential expression with

DESeq software. Using a porcine model of SCI, we demonstrate miRNA profiles in CSF and serum samples during acute and sub-acute stages after SCI. Our results reveal pronounced trends in miRNA expression and their potential target interactions provide valuable insight into the molecular mechanisms of SCI. We are currently also examining serum and CSF samples from human SCI patients collected at identical post-injury time points for a direct comparison of miRNA responses to SCI between the two species. This characterization is important to establish whether biomarkers of SCI found in pigs can be transferred to humans and visa-versa.

Disclosures: **S.S. Tigchelaar:** None. **F. Streijger:** None. **S. Sinha:** None. **N. Manouchehri:** None. **K. So:** None. **I. Malencia:** None. **A. Courtright:** None. **T. Beecroft:** None. **K. Van Keuren-Jensen:** None. **C. Nislow:** None. **B. Kwon:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.11/H10

Topic: C.10. Trauma

Support: NY State Empire Clinical Research Investigator Program

Title: Characterization of circulating monocytes in individuals with acute spinal cord injury

Authors: ***O. BLOOM**[1], R. MONAHAN[2], K. GIBBS[1], A. PAPATHEODOROU[2], A. STEIN[3], M. BANK[4];
[1]Feinstein Institute, Hofstra North Shore LIJ Sch. of Med., Manhasset, NY; [2]Feinstein Inst., Manhasset, NY; [3]Hofstra North Shore LIJ Sch. of Med., Manhasset, NY; [4]North Shore Univ. Hosp., Manhasset, NY

Abstract: Introduction: Acutely after traumatic spinal cord injury (SCI), inflammation exacerbates neuronal loss and promotes secondary tissue damage. We and others recently discovered a subset of elevated circulating inflammatory mediators in individuals with acute or chronic SCI (Bank et al 2015, Stein et al 2013). Conversely, immune depression has also been noted in individuals with acute SCI and neurological recovery is inversely correlated with infections after SCI (Riegger et al 2009, Failli et al 2012, Kopp et al 2013). In the present study, our objective was to profile the distribution and activation status of circulating monocyte and dendritic cell subsets in individuals with acute SCI. Uninjured individuals of the same age range and gender distribution served as controls. Methods: This prospective, IRB-approved, observational pilot study of adult SCI participants was performed in an academic medical center. "Acute" SCI was defined as <1 week from initial injury. PBMCs were isolated from blood by Ficoll density gradient. Multicolor flow cytometric analysis of PBMCs was performed to examine the number, percentage and phenotype of circulating monocyte and dendritic cell

subsets, using gating schemes recommended by the Human Immunology Project (Maecker et al 2012). Activation status was measured by cell surface expression of MHCII/HLA-DR, as indicated by the median fluorescence intensity (MFI) of a fluorescent anti-HLA-DR antibody conjugate. Results: Uninjured (N=7) and SCI (N=3) participants included 2 females in each group. Uninjured and SCI participants were of similar ages (mean±sem, range): (52±4, 45-57 and 53±5, 48-58 years) ($P<0.7$). Mechanisms of injury for SCI participants were: Fall (n=1), MVA (n=2). All SCI individuals had a cervical level injury (C4-C6) and two had neurologically incomplete injuries. In this initial small number of participants, we did not observe differences in the relative distribution of CD3- CD56- circulating monocyte and dendritic cell subsets in SCI as compared to uninjured individuals. However, in acute SCI, we did observe statistically significant decreases in activation status of several cell subsets, as indicated by decreased HLA-DR cell surface expression: CD11c+ CD16- dendritic cells ($P<0.004$), CD14++ CD16- classical monocytes ($P<0.004$), and CD14+ CD16+ non-classical monocytes ($P<0.02$). This data confirms and extends previous studies of circulating immune cell populations in acute SCI (Riegger et al 2009, Kopp et al 2013). Studies are ongoing to confirm and extend these findings in additional participants.

Disclosures: **O. Bloom:** F. Consulting Fees (e.g., advisory boards); ad hoc reviewer, Craig Neilsen Foundation. **R. Monahan:** None. **K. Gibbs:** None. **A. Papatheodorou:** None. **A. Stein:** F. Consulting Fees (e.g., advisory boards); consultant, Craig Neilsen Foundation, member, Data Monitoring Safety Committee, StemCells, Inc.. **M. Bank:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.12/H11

Topic: C.10. Trauma

Support: Halbert Chair in Neural Repair and Regeneration

Title: Circulating microRNAs reflect neural dysfunction in patients with cervical spondylotic myelopathy (CSM): Implications for a novel biomarker of disease pathobiology

Authors: *A. M. LALIBERTE[1], S. K. KARADIMAS[1], S. KALSI-RYAN[2], A. NOURI[2], E. MASSICOTTE[2], M. G. FEHLINGS[2,3];
[1]Genet. and Develop., Univ. of Toronto, Toronto, ON, Canada; [2]Spine Program, [3]Develop. and Genet., Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Introduction: While increasing evidence points to a beneficial role for surgical decompression in CSM, approximately 5% of patients with CSM sustain neurological decline following decompression with risk factors remaining unclear. Additionally, patients with

significant cord compression on MRI but minimal clinical symptoms represent a significant management challenge. We hypothesize, based on recent work from preclinical animal models, that circulating microRNAs (miRs) could reflect the pathobiology of CSM and thus could serve as biomarkers of disease progression. **Methods**: Thirty CSM and ten healthy subjects were recruited for the initial screening study with another 40 subjects recruited for validation. Blood plasma was collected from all subjects, and 179 miRs were screened using the Exiqon miRCURY Plasma PCR platform. The Normfinder algorithm was used to determine optimal normalization for the dataset. Subjects were divided into healthy, mild CSM, moderate/severe CSM groups (based on mJOA scale) and ANOVA was used to determine significant differences. Logistic regression models were created to distinguish healthy versus CSM subjects, as well as mild versus moderate/severe CSM cases. Model validation was performed using an 80 replicate bootstrap re-sampling procedure. **Results**: The mean age and mJOA scores were 53.3±10.8/15.9±0.8, 60.1±9.4/12.4±1.4, and 51.7±10.9 in mild CSM, moderate/severe CSM and healthy subjects, respectively. The gender ratio was 1:1 in healthy and CSM groups. Eight miRs had significant ($p<0.05$) expression differences between groups. Four of those miRs (let-7f-5p, miR-34a, let-7c, miR-154-5p) contributed to the logistic regression models. These models discriminated well between healthy and CSM subjects (let-7f-5p [OR= 0.106], miR-34a [OR=0.232], let-7c [OR=27.4], AUC = 0.837) and between mild CSM and moderate-severe CSM patients (let-7f-5p [OR=0.040] and miR-154-5p [OR=5.5], AUC = 0.930). Model performance with the bootstrap replicates decreased marginally in discrimination of healthy versus CSM subjects (AUC=0.713), but remained high for CSM severity discrimination (AUC=0.889) **Conclusions**: The results reported herein demonstrate that plasma miR expression can predict the presence and severity of CSM. Based on previous work in the preclinical model, it is plausible that these miRs are related to the underlying ischemic and inflammatory mechanisms driving myelopathy. Future work will focus on assessing potential of miRs in identifying patients at risk for neurological decline following decompression and those with cord compression and minimal clinical symptoms that are at risk for disease progression.

Disclosures: **A.M. Laliberte:** None. **S.K. Karadimas:** None. **S. Kalsi-Ryan:** None. **A. Nouri:** None. **E. Massicotte:** None. **M.G. Fehlings:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.13/H12

Topic: C.10. Trauma

Support: Craig H. Neilsen Foundation grant 224308

NIH grant NS067092

NIH grant NS069537

NIH grant NS079030

NIH grant NS032000

NIH grant NS088475

Wings for Life Foundation grant WFLU008/12

Title: Leveraging big-data analytics for reproducibility testing in preclinical spinal cord injury research

Authors: *J. L. NIELSON[1], C. F. GUANDIQUE[1], A. W. LIU[1], C. A. TOVAR[2], M. S. BEATTIE[1], J. C. BRESNAHAN[1], A. R. FERGUSON[1];
[1]Brain and Spinal Injury Ctr., Univ. of California San Francisco, San Francisco, CA; [2]Neurosci., Ohio State Univ., Columbus, OH

Abstract: In recent years, the field of spinal cord injury (SCI) research has identified multiple replication failures which could result in an inability to translate findings into standards of patient care. Several theories have emerged about the cause of the inability to replicate, both from the preclinical and clinical perspectives. One emerging theory implicates methods that data are collected, analyzed and selected for publication. Our group has developed a novel approach to address these concerns, known as syndromics, applying large scale bioinformatics and multivariate statistics to identifying complex interactions in pathophysiology using the full set of multidimensional data gathered for each individual test subject. We have compiled such data into the VISION-SCI database, with raw data from over 3000 mice, rats and monkeys donated by members of the SCI research community. Application of data-driven analytics to preclinical health records identified significant syndromic impact of perioperative hypertension (MAP) as a major predictor of poor neurological recovery. Here, we apply data from the VISION-SCI database to test the reproducibility of the relationship between MAP and neurological recovery in adult male and female rats receiving a range of thoracic bilateral SCI contusions (T9; weight-drop impactor; 12.5, 25, 50mm). We applied topological data analysis (TDA), which deploys ensemble machine learning in multidimensional space to heterogeneous, complex big-data on measures of perioperative vitals (body temperature, heart rate, blood pressure), blood gases, weight monitoring, bladder care, locomotor functional recovery (BBB, 1-6 weeks) and terminal tissue sparing (6 weeks) (N=334). TDA revealed a data-driven, syndromic relationship between perioperative care and locomotor recovery on a subset of the animals (N=72). Cross-validation of TDA-identified patterns was performed on the remaining animals (N=262) using an analytical workflow of TDA, post-hoc repeated-measures general linear model (GLM) and bivariate correlations. TDA identified syndromic dysfunction in BBB recovery, significantly predicted by hypertensive episodes (MAP>140mmHg) during SCI operation. Cross-validation in the independent dataset revealed a similar significant difference in BBB recovery inversely predicted by MAP. GLM on BBB recovery revealed MAP significantly predicted locomotion in both datasets, and correlational analyses confirmed an inverse relationship. Together the data indicate

that our big-data analytical model identified and replicated the finding that upper extremes of perioperative MAP predict poor recovery following SCI.

Disclosures: **J.L. Nielson:** None. **C.F. Guandique:** None. **A.W. Liu:** None. **C.A. Tovar:** None. **M.S. Beattie:** None. **J.C. Bresnahan:** None. **A.R. Ferguson:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.14/H13

Topic: C.10. Trauma

Support: GHNF

CIHR

CDRF

DOD

Title: Dietary strategies following spinal cord injury: Ketogenic Diet and Modified Adkins Diet with oral administration of ketone supplementation

Authors: *F. STREIJGER[1], K. WU[1], O. JANG[1], W. T. PLUNET[1], J. LIU[1], W. TETZLAFF[1,2]; [1]ICORD, UBC, Vancouver, BC, Canada; [2]Dept. of Zoology, UBC, Vancouver, BC, Canada

Abstract: Contrary to our current clinical practice, we reported that feeding a low carbohydrate, high fat ketogenic diet (KD) after spinal cord injury (SCI) improved reaching ability that involves fine manipulation of the distal muscles of the digits and supination in rats. Despite these intriguing results, potential clinical application of KD has been significantly hampered by poor tolerability, its strictness, lack of palatability, and potential side effects after long-term consumption. A safer and more tolerable diet therapy would be highly desirable, as it would extend the availability to more patients with SCI. The Modified Atkins Diet (MAD) was designed to mimic the effects on ketosis and seizures of KD, but with less restrictiveness. Unlike the standard Atkins Diet, MAD does not restrict calories, allowing unlimited protein and fat intake, and is more lenient with the use of estimations of portion sizes. Evidence suggesting that the MAD may exhibit similar beneficial properties as the traditional KD is accumulating in other fields. Therefore, the objectives of this study are 1) to determine whether a more palatable MAD would be similarly effective as KD in the SCI setting and 2) to investigate whether the beneficial effects of KD or MAD can be further enhanced by intermittent bolus gavage feeding with oral ketone esters. Here, we used a cervical hemi-contusion model. Following SCI surgery, animals were placed on a carbohydrate-based standard diet, KD, or MAD with or without ketone ester supplementation during the first 3-days of treatment. Forelimb function was assessed using a

battery of tests to assess manual dexterity and distal muscle activation in the forelimb. Our data suggest that ketone supplementation results in a more effective induction of effective ketone levels in the blood compared to KD or MAD alone. Oral administration of ketones effectively induced a rapid and sustained ketosis within 24 hours in both the KD and MAD group. Supplementation of KD with oral ketones increased blood ketones to levels around 3.2 mmol/L, which is almost 2 times higher than we observe after KD consumption in rats without supplementation. Behavioural analyses are currently performed and will be presented. In conclusion, the data generated by this research will further our understanding of the unique nutrition requirements of patients with SCI in order to improve neurological outcome. The possible improvement from this simple dietary intervention would improve quality of life of the affected individuals and safe significant health care costs.

Disclosures: F. Streijger: None. **K. Wu:** None. **O. Jang:** None. **W.T. Plunet:** None. **J. Liu:** None. **W. Tetzlaff:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.15/H14

Topic: C.10. Trauma

Title: Effective gene silencing in brain and spinal cord *in vivo* models mediated by lipid nanoparticle technology

Authors: O. SEIRA[1], J. LIU[1], A. ANSARI[2], D. ZWAENEPOEL[2], C. L. WALSH[2], A. THOMAS[2], T. LEAVER[2], A. WILD[2], J. R. TAYLOR[2], E. RAMSAY[2], P. CULLIS[3], *W. TETZLAFF[4];
[1]Zoology Department. Univ. of British Columbia, Intl. Collaboration on Repair Discoveries (ICORD), Vancouver, BC, Canada; [2]Precision NanoSystems Inc., Vancouver, BC, Canada; [3]Dept. of Biochem. & Mol. Biol., [4]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Lipid nanoparticles (LNP) are the leading systems for *in vivo* delivery of short interfering RNA (siRNA) for therapeutic applications. The lack of an appropriate protocol to administer LNP into the brain and spinal cord has limited the investigation of their potency to silence neuronal genes *in vivo*. Here, we attempt to bridge this gap in neuroscience research by describing the use of siRNA-LNP to efficiently knockdown gene expression in brain and, for the first time, in spinal cord *in vivo* models. The effectiveness of the siRNA-LNP system was assessed in the brain by direct injection into the cortex. LNPs were seen to be present in cortical neurons, where delivery of the siRNA against PTEN exerted the desired changes in gene expression. Subsequently, LNPs were injected at the site of cervical spinal cord injury and a decrease in expression of the target PTEN was observed in the vicinity of the injury/injection site

10 days later. Moreover, the presence of LNPs in the neurons of the red nucleus located in the brainstem, suggests that the lipid nanoparticles are taken up by axons and retrogradely transported. With no previous reports on this phenomenon, this study reflects the potential of siRNA-LNPs in advancing our understanding of the central nervous system. Furthermore, the ability of this relatively novel siRNA-delivering LNP technology to successfully affect gene silencing in brain and spinal cord *in vivo*, presents a promising prospect for the development of new gene therapies to treat neurological disorders.

Disclosures: O. Seira: None. **J. Liu:** None. **A. Ansari:** None. **D. Zwaenepoel:** None. **C.L. Walsh:** None. **A. Thomas:** None. **T. Leaver:** None. **A. Wild:** None. **J.R. Taylor:** None. **E. Ramsay:** None. **P. Cullis:** None. **W. Tetzlaff:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.16/H15

Topic: C.10. Trauma

Support: NIH Grant 5T32GM008620

NIH Grant NS047718

Generous private donations

Title: AAV-mediated conditional deletion of PTEN in the adult sensorimotor cortex causes robust hypertrophy of cortical motoneurons

Authors: *E. A. GUTILLA[1], O. STEWARD[2];
[1]UC IRVINE SCHOOL OF MEDICINE, Irvine, CA; [2]ANATOMY & NEUROBIOLOGY, UC IRVINE, IRVINE, CA

Abstract: Previous studies have reported neuronal hypertrophy and cortical enlargement following deletion of the phosphatase and tensin homolog on chromosome 10 gene (PTEN) in developing mice and in newborn neurons in adult mice. Here, we show that deletion of PTEN in adult sensorimotor triggers remarkable enlargement of individual cortical motoneurons which give rise to the corticospinal tract (CST) as well as alterations in cortical lamination. Using the same approach we have previously used to promote axon regeneration and functional recovery following spinal cord injury, AAV-Cre was injected locally into the sensorimotor cortex of adult (8 week old) mice with a lox-P flanked exon 5 of the PTEN gene. Control animals received intracortical injections of AAV-GFP. Mice were allowed to survive for up to one year following AAV injections. One group of mice with focal PTEN deletion received bilateral fluorogold injections at cervical level 5 of the spinal cord one week prior to perfusion to retrogradely label

the cells of origin of the CST. Brain sections were immunostained for PTEN to identify the region of PTEN deletion and for phosphorylated ribosomal protein S6 (pS6), which is a marker for mTOR activation. In sections immunostained for PTEN, the area of deletion was marked by a complete absence of immunostaining in a region about 1mm in diameter. Immunostaining for pS6 revealed intensely stained neuronal cell bodies and processes within the region of PTEN deletion; pS6-positive pyramidal neurons in layer V were obviously larger than PTEN-positive neurons in other areas of the cortex. Neurons in layer V that were retrogradely labeled following fluorogold injections into the spinal cord were substantially larger in the area of PTEN deletion than in neighboring layer V neurons with intact PTEN expression. Our results indicate that deleting PTEN re-initiates the ability for robust neuronal growth that is normally restricted to the developmental state. These finings suggest the potential usefulness of PTEN interference in adults to protect and repair vulnerable neurons from the effects of aging traumatic injury, or neurodegenerative disease.

Disclosures: E.A. Gutilla: None. **O. Steward:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.17/H16

Topic: C.10. Trauma

Support: VARRD

The Gordon Project to Cure Clinical Paralysis

Title: Evoked potential analysis of hmsc and anastomosis mediated functional recovery for chronic thoracolumbar sci

Authors: *X. ZENG[1,2], D. YU[1,2], J. E. ANDERSON[1,2], Z. ALJUBOORI[1,2], R. D. ZAFONTE[3], Y. D. TENG[1,2,3];
[1]Dept. of Neurosurg., Brigham & Women's Hospital/Harvard Med. Sch., Boston, MA; [2]Div. of SCI Res., VA Boston Healthcare Syst., Boston, MA; [3]Physical Med. and Rehabil., Spaulding Rehabil. Hospital/Harvard Med. Sch., Boston, MA

Abstract: We previously reported that anastomosis/neurotization could significantly improve locomotion in rats with thoracolumbar contusion spinal cord injury (SCI). The benefits included enhancing coordinated hindlimb functions of open field locomotion and incline plane performance, and sensorimotor recoveries evaluated by spinal reflex tests. Post-neurotization recovery neurobiology mechanisms comprised enrichment of intraspinal cord synapses and strengthened propriospinal projection connectivity across the contusion site. To further assess specific neural circuit(s) involved in locomotion recovery, we performed a pilot study on evoked

potentials in chronic SCI rats that demonstrated persistent locomotion improvement (i.e., ≥ 12 months) following neurotization treatment. Briefly, female SD rats (220-235g) received T13-L1 moderate contusion (10gx25mm) and T12-L3 neurotization at either 1 week (subacute) or 13 weeks (chronic) after SCI (n=4/each study). For rats with chronic SCI, neurotization was done 7-10 days following human mesenchymal stromal stem cell (hMSC) injection (50K cells/µl; 1µl injection at 1 mm rostral and caudal to the epicenter, respectively and 2µl into the injury site; total: 200K cells/rat; n=4/group). Behavior tests were performed weekly for 10 months in order to confirm sustainability of locomotion recovery. Based on our previous finding, we hypothesized that the regained locomotion capability might be partially facilitated by serotonergic modulation. We therefore administered 5-HT1A and 2A/2C agonists and/or antagonists intraperitoneally or intrathecally to examine their effects on hindlimb function. The results replicated data we reported before, showing that the locomotion recovery could be additionally boosted by treatment with 5-HT1A and 2A/2C agonists acting specifically via corresponding receptors. We next measured cortical motor evoked or peripheral nerve (C7) evoked potentials (EPs) recorded at loci either rostral (T6-T7) or caudal (L3-L4) to the injury epicenter to determine which neural circuit(s) contributed to reactivation of the central pattern generation (CPG). The EP outcomes, together with neural tracing data, suggest that T12-L3 neurotization following hMSC implantation provides a multimodal approach to functional recovery for chronic thoracolumbar contusion SCI. (Supported by VARRD and the Gordon Project to Cure Clinical Paralysis)

Disclosures: X. Zeng: None. **D. Yu:** None. **J.E. Anderson:** None. **Z. Aljuboori:** None. **R.D. Zafonte:** None. **Y.D. Teng:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.18/H17

Topic: C.10. Trauma

Support: US Department of Veterans Affair Rehab R&D Merit Review grants B1005-R

US Department of Veterans Affair Rehab R&D Merit Review grants B78071

US Department of Veterans Affair Rehab R&D Merit Review grants B6570R

Title: Safety and feasibility of the use of tms on the cervical spinal cord injury site: a radio-telemetric study of cardiovascular and brain activity

Authors: *P. K. BOSE[1,2,3], J. HOU[1,2], R. NELSON[1], M. H. MODARRES[1], F. J. THOMPSON[1,2,4];

[1]North Florida/South Georgia VAMC, Gainesville, FL; [2]Dept. of Physiological Sci., [3]Dept. of Neurol., [4]Dept. of Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Cervical spinal cord injury (cSCI) is frequently a devastating injury that can result in a broad range of life-long disabilities. Development of safe and effective therapies for these long-term disabilities is urgently needed. Our recent work using a combination of treadmill locomotor exercise (Tm) with Transcranial magnetic stimulation (TMS) therapy yielded significant locomotor improvement that was greater than either treatment tested alone (Hou et al, 2014). The present studies were conducted to provide neurological and cardiovascular measures to serve as quantitative safety assessments of the influence of TMS on brain and cardiovascular activity. We utilized implanted telemetry devices to measure blood pressure (BP), heart rate (HR), and brain activity (EEG). The data were acquired for 7 consecutive days before injury, 7 consecutive days after SCI, and after each therapeutic intervention. Beginning at post-cSCI wk-3, animals received Tm and TMSsc as an individual or combined therapy using single pulse TMSsc (sTMSsc) (Hou et al., 2014) or rTMS (15 Hz, 30-70% TMS power). Data were acquired just before intervention and within 2 minutes after interventions, and continued for an hour to test the immediate effects of treatments on BP, HR, and EEG. The BP and HR data and EEG were acquired and analyzed by a digital signal acquisition and analysis system (DSI Ponemah Physiology Platform, Version 5.00) and NeuroScore, 2.1 respectively. Power spectral decomposition of EEG records computed the absolute power for all frequencies from 0.5 to 45 cycles/second (Hz) (Welch's averaged periodogram) on windowed (Hanning) 2-second consecutive segments of EEG records (overlapped by 1 second), which produced EEG power vs. frequency levels at a resolution of 0.5 Hz. We computed quantities that represented the power levels over various frequency bands as follows: δ (0.5 - 3 Hz), θ (3.5 - 7 Hz), α (7.5 - 11.5 Hz), β (13 - 28 Hz), and γ (39 - 45 Hz). We also computed the power for 2 sub-segments of β band: β1 (13 - 17.5 Hz), and β2 (18 - 22 Hz). There were no significant changes in BP and HR in pre- and post-cSCI conditions as well as pre- and post-intervention with sTMS. However, rTMS (with or without Tm exercise) produced significantly elevated BP and HR. cSCI animals showed significantly elevated α band in averaged EEG absolute and relative powers when compared to that of pre-injured data. Interestingly, sTMSsc or rTMS normalized this difference. However, power of the γ band was significantly increased after rTMS with or without Tm. These preliminary studies indicated that sTMSsc does not produce detectable alterations in the electrophysiological patterns of cardiovascular and/or brain activity.

Disclosures: P.K. Bose: None. **J. Hou:** None. **R. Nelson:** None. **M.H. Modarres:** None. **F.J. Thompson:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.19/H18

Topic: C.10. Trauma

Support: NIH Grant NS052290

NIH Grant NS052290-06S1

NIH Grant NS050243

NIH Grant NS059622

ISDH Grant A70-2-079609

ISDH Grant A70-9-079138

ISDH Grant A70-5-0791033

Title: Lipidomic analysis of spinal cord injury: cardiolipin loss and peroxidation mediated by cPLA2 activation

Authors: ***N.-K. LIU**[1], L.-X. DENG[1], M. WANG[2], Q.-B. LU[1], X.-B. WU[1], C. WANG[2], X. HAN[2], X.-M. XU[1];
[1]Indiana Univ., Indianapolis, IN; [2]Sanford-Burnham Med. Res. Inst., Orlando, FL

Abstract: Traumatic spinal cord injury (SCI) is prevalent in the United States, with more than 12,000 cases every year. Mitochondrial dysfunction and neuronal apoptosis have been shown to play a key role in SCI. Alteration (loss and peroxidation) of cardiolipin (CL) is emerging as an important factor in mitochondrial dysfunction and in the initial phase of the apoptotic process. However, whether CL alteration occurs following SCI and, if so, its role in mediating SCI remains unclear. In the present study, we examined alteration of CL following SCI using mass-spectrometry-based lipidomics. Pharmacological, genetic, molecular, biochemical, and immunohistological approaches were used to explore the role and mechanism of CL alteration after SCI. The results showed that the content of CL was significantly reduced at 3 and 24 h after SCI while lyso-cardiolipin (lyso-CL) was increased only at 24 h after the injury. Over 50 distinct CL molecular species were readily identified. Of them, 50% were significantly reduced after SCI. Additionally, mitochondrial 4-HNE also increased at 3 and 24 h after SCI. *In vitro* experiments showed that activation of cPLA2 resulted in CL loss, leading to mitochondrial dysfunction and neuronal death, which were substantially reversed by AACOCF3, a cPLA2 inhibitor. *In vivo* experiments showed that cPLA2 activation was increased in the mitochondria after SCI. Remarkably, blocking cPLA2 pharmacologically with AACOCF3 reduced CL loss, leading to decrease in mitochondrial dysfunction, cytochrome c release, and neural apoptosis after SCI. Genetic deletion of cPLA2 also inhibited CL loss, resulting in neuroprotection after SCI. These findings collectively suggest that CL alteration is an early response following SCI and such SCI-induced CL alteration was mediated by cPLA2 activation, at least in part. Thus, CL alteration may play an important role in the pathogenesis of SCI, and as such could be an attractive therapeutic target for ameliorating secondary SCI.

Disclosures: **N. Liu:** None. **L. Deng:** None. **M. Wang:** None. **Q. Lu:** None. **X. Wu:** None. **C. Wang:** None. **X. Han:** None. **X. Xu:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.20/H19

Topic: C.10. Trauma

Support: Merit Review Award BX002343

Title: Persistent inflammatory response to spinal cord injury leads to decrease of mRNA editing through down-regulation of Adar2

Authors: ***S. DRACHEVA**[1,2], A. F. DI NARZO[2], A. KOZLENKOV[2], Y. GE[2], B. ZHANG[2], C. CARDOZO[1], E. KOONIN[3], L. SANELLI[4], Z. MAY[4], Y. LI[4], K. FOUAD[4], D. BENNETT[4]; [1]Dept. Psychiat, James J. Peters VA Med. Ctr., Bronx, NY; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Natl. Ctr. for Biotech. Information, Natl. Library of Medicine, Natl. Inst. of Health,, Bethesda, MD; [4]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Spasticity is a common sequella of spinal cord injury (SCI) and is a major complaint of injured individuals. It involves simultaneous contractions of many muscles, which are triggered by brief sensory stimuli (e.g., cutaneous inputs). Spastic paralysis has been observed in ~ 80% of individuals with traumatic SCI. Treatment of spasticity with conventional antispastic drugs (e.g., baclofen,) is often not adequate, or is not tolerated because of adverse side effects such as drowsiness and weakness. We have recently showed that SCI leads to a decrease in mRNA editing of serotonin receptor 2C (5-HT2CR) contributing to post-SCI spasticity. Editing of 5-HT2CR is catalyzed by RNA-specific adenosine deaminases (Adar1-2). The extent of editing correlates with 5-HT2CR functional activity: more highly edited isoforms exhibit the least function. Editing substantially increases the functional plasticity of this key neurotransmitter receptor and is thought to contribute to homeostatic mechanisms in neurons. In this work we studied post-SCI mRNA editing and genome-wide RNA expression in a chronic spinal rat model of spasticity using massively parallel sequencing. We detected that the decrease in 5-HT2CR editing is caused by down-regulation of Adar2 and that editing of at least one other Adar2 target, potassium channel Kv1.1, is decreased after SCI. Bayesian network analysis of genome-wide transcriptome data indicates that down-regulation of Adar2 (1) is triggered by persistent inflammatory response to SCI that is associated with activation of microglia and (2) results in changes in neuronal gene expression that are likely to contribute both to post-SCI restoration of neuronal excitability and muscle spasms. The results of this work start to elucidate the specific molecular mechanisms that are associated with alterations of RNA editing in SCI and ultimately could lead to effective anti-spasticity treatments.

Disclosures: **S. Dracheva:** None. **A.F. Di Narzo:** None. **A. Kozlenkov:** None. **Y. Ge:** None. **B. Zhang:** None. **C. Cardozo:** None. **E. Koonin:** None. **L. Sanelli:** None. **Z. May:** None. **Y. Li:** None. **K. Fouad:** None. **D. Bennett:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.21/H20

Topic: C.10. Trauma

Support: Internal - UAB Comprehensive Neuroscience Center

Title: HuR modulates the inflammatory response in astrocytes following spinal cord injury

Authors: ***T. KWAN**[1], C. L. FLOYD[2], P. H. KING[3];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Physical Med. and Rehabil., [3]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Background/hypothesis: A rapid induction of inflammatory cytokines in glial cells is generated within minutes following traumatic spinal cord injury (SCI). This initiates a cascade of events that contributes to secondary injury to the spinal cord over time. The RNA binding protein human antigen R (HuR) is reported to bind and stabilize the mRNAs of multiple inflammatory cytokines and chemokines. We hypothesize that reducing HuR expression or activity will dampen the inflammatory response elicited by astrocytes. An increase in HuR expression/activity will result in the opposite. The ability of HuR to modulate the expression of multiple inflammatory cytokines could have implications on damage resulting from secondary injury from SCI and thus, functional recovery. Methods: Primary cortical astrocytes were subjected to a mechanical stretch injury to emulate the viscoelastic forces that occur during trauma to CNS tissue. HuR expression in astrocytes was either knocked down using siHuR or inhibited using MS-444, a small molecule HuR inhibitor. mRNA and protein for the cytokines/chemokines TNFα, IL-1β, IL-6, CXCL-1, LIF and CCL2 were measured 24 hours after stretch injury. *In vivo*, neurons were counted in female FlagHuR-astrocyte transgenic mice and compared against WT mice 24 h after a midthoracic contusion injury. FACS was used to measure neutrophil and microglia/macrophage populations in transgenic and WT mouse spinal cords 24h post-injury. Results: In our *in vitro* model, HuR knockdown and MS-444 treatment reduced the expression of a number of cytokines/chemokines at both the mRNA and protein level. *In vivo*, spinal cords in the transgenic mice overexpressing HuR in astrocytes had fewer neurons at the epicenter of injury 24 hours after SCI and a trend toward increased neutrophil invasion. Conclusion: 1. Knockdown/inhibition of HuR attenuated inflammatory cytokine induction in mechanically stretched astrocytes. 2. Transgenic HuR expression in astrocytes enhanced neuronal loss. 3. The

data suggests that HuR may be a viable target in controlling inflammation and secondary damage in the context of spinal cord injury.

Disclosures: **T. Kwan:** None. **C.L. Floyd:** None. **P.H. King:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.22/H21

Topic: C.10. Trauma

Support: NSERC

Title: Activated astrocytes modulate survival and fate specifications of adult spinal cord neural precursor cells

Authors: ***C. HART**, S. M. DYCK, S. KARIMI-ABDOLREZAEE;
Physiol. and Pathophysiology, Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Neural stem/progenitor cells (NPCs) that reside in the adult spinal cord become activated following spinal cord injury (SCI) and contribute to generation of new glial cells. Despite their intrinsic ability to generate both oligodendrocyte and astrocytes, adult NPCs predominantly differentiate into astrocytes in the post-SCI milieu, with only a limited number giving rise to oligodendrocytes. This evidence implicates a role for the injury microenvironment in influencing the regenerative response of spinal cord NPCs. Astrocytes are a key modulator of their microenvironment both in the normal and injury conditions. Following SCI, resident astrocytes undergo drastic changes that result in their transition into an activated inhibitory phenotype. Here, we investigated how reactive astrocytes influence the cellular properties of spinal cord derived NPCs in their post-injury environment using an *in vitro* model of reactive astrogliosis. Rat primary astrocyte cultures were activated by lipopolysaccharide (LPS) and transforming growth factor beta (TGF-β). Astrocyte reactivity was confirmed by increased expression of chondroitin sulfate proteoglycans (CSPGs) and proinflammatory cytokines in the conditioned media of astrocytes treated by TGF-β and LPS, respectively. Primary NPCs were harvested from the spinal cord of adult mice and propagated through passaging. Dissociated NPCs were subject to astrocyte conditioned media (ACM) of normal, LPS and TGF-β treated astrocytes. Using various cellular and molecular *in vitro* assays, we found that exposure to LPS-treated ACM markedly decreased NPCs survival and reduced their capacity for oligodendrocyte differentiation compared to the control normal ACM or serum free media. Importantly, LPS-treated ACM favored astrocyte differentiation of NPCs. We found the same but smaller effect in spinal cord NPCs subject to TGF-β-treated ACM. Interestingly, our analysis showed that increased levels of CSPGs in LPS and TGF-β treated astrocytes was in part responsible for these

inhibitory effects since removal of CSPGs with chondroitinase ABC was able to reverse some of these effects. Our data suggests the impact of reactive astrocytes in regulating the properties of NPCs in their post-injury niche. Identification of astrocyte-derived factors involved in NPCs modulation can potentially improve the regenerative response of NPCs in injury condition. Supported by Natural Sciences and Engineering Council of Canada (NSERC).

Disclosures: C. Hart: None. **S.M. Dyck:** None. **S. Karimi-Abdolrezaee:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.23/H22

Topic: C.10. Trauma

Support: NIH NS050243

NIH NS059622

DOD CDMRP W81XWH-12-1-0562

DVA 1I01BX002356-01A1

Craig H Neilsen Foundation 296749

Title: Dendritic plasticity of layer V pyramidal neurons in the primary motor cortex after lesions to the pyramid and thoracic spinal cord

Authors: X. LIN[1], T. ZHAO[2], X. GAO[1], W. XIONG[1], S. ZHAO[1], W. WU[1], X. PING[1], S. LIN[3], X. JIN[1], J. CHEN[1], W. GAN[4], *X. M. XU[5];
[1]Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Gen. Hosp. of Jinan Military Region, Jinan, China; [3]Beijing Inst. of Basic Med. Sci., Beijing, China; [4]New York Univ. Sch. of Med., New York, NY; [5]Neurolog. Surgery, Indiana Univ., Indianapolis, IN

Abstract: Axons originating from layer V pyramidal neurons of the primary motor cortex (M1) form the corticospinal tract (CST) that descends mainly to the ipsilateral medulla pyramid and contralateral spinal cord. Following injury, these neurons may undergo structural remodeling that affects spontaneous recovery. Here we investigated structural changes in the fore- (M1F) and hind-limb (M1H) regions of M1 as well as functional outcomes following 4 types of injuries that all induced CST axotomy: ipsilateral pyramidotomy (PX), contralateral dorsal funiculotomy (FX), contralateral spinal hemisection (HX), and complete spinal cord transection (TX) in adult rats and mice. The PX was performed at the medullary pyramid and all other injuries were performed at the 9th thoracic spinal level (T9). Combined neurobehavior (Treadscan, Basso-Beattie-Bresnahan [BBB] locomotor scale, grid walking, Morris water maze),

electrophysiological (motor-evoked potentials [MEPs]), histological (Golgi-Cox impregnation), and imaging (2-photon confocal imaging) assessments were performed to determine the relationships among motor function, pathway conductivity, and cortical plasticity. Compared to the sham-operated controls, animals receiving different injuries exhibited injury site- and severity-specific deficits and MEP alterations in fore- and hind-limb functions. Among all injury types, TX was associated with the worst spontaneous functional recovery. Two-photon imaging showed that, as early as 1 week post-PX, turnover of apical dendritic spines occurred and the rates of spine formation and elimination were significantly higher in M1F, compared to normal controls. However, PX had no significant effect on spine dynamics in M1H. Both FX and HX increased spine formation of apical dendrites whereas TX had no significant effect on spine dynamics in M1F. In the M1H region, spine elimination of apical dendrites was significantly increased following all injuries at T9. Significantly increased spine formation was found in the FX and HX but not the TX group as compared to the control. Golgi-Cox staining showed that, in the HX group, the dendritic surface area (SA) of both basal and apical dendrites was significantly increased and that, in the TX group, the total length and SA were significantly decreased. These results underscore the profound influences of synaptic structural plasticity on spontaneous recovery that occur overtime after axotomy of the layer V cortical neurons.

Disclosures: **X. Lin:** None. **T. Zhao:** None. **X. Gao:** None. **W. Xiong:** None. **S. Zhao:** None. **W. Wu:** None. **X. Ping:** None. **S. Lin:** None. **X. Jin:** None. **J. Chen:** None. **W. Gan:** None. **X.M. Xu:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.24/H23

Topic: C.10. Trauma

Support: DOD, SCIRP Award number W81XWH-12-1-0563

Title: The role of L-selectin in leukocyte recruitment and secondary pathogenesis following spinal cord injury

Authors: *D. A. MCCREEDY[1,2], C. J. SONTAG[2], S. M. LEE[2], A. F. MARTINEZ[2], T. M. FANDEL[2], S. D. ROSEN[3], L. J. NOBLE-HAEUSSLEIN[2];
[1]Gladstone Inst., San Francisco, CA; [2]Dept. of Neurolog. Surgery, [3]Dept. of Anat., Univ. of California, San Francisco, CA

Abstract: L-selectin, a receptor expressed on all leukocyte classes, is implicated in adhesive and signaling roles in the recruitment of myeloid cells to sites of inflammation. Spinal cord injury (SCI) produces prolonged inflammation leading to secondary neural damage and expansion of

the spinal cord lesion area. Given its fundamental role in inflammation, L-selectin may be integral to secondary pathogenesis in the injured spinal cord. Using L-selectin knock-out (KO) mice, we observed improved neurological recovery and greater white matter sparing compared to wild-type (WT) controls that corresponded to reduced oxidative stress and degradation of myelin basic protein in the acutely injured cord. WT mice treated with diclofenamic acid (DFA), an FDA approved non-steroidal anti-inflammatory drug with high L-selectin sheddase activity, immediately post-injury showed improved recovery on the Basso Mouse Scale (BMS) and enhanced white matter preservation compared to vehicle controls. L-selectin KO mice, treated with DFA, showed similar robust levels of behavioral improvement and white matter sparing to that of vehicle treated KO mice, suggesting that the effect of DFA is specific to its L-selectin sheddase activity. To determine if shedding of L-selectin affected early leukocyte recruitment, we performed flow cytometry at 24 hours post-injury on spinal cords from WT mice treated with DFA. CD45+/Gr1+ and CD45+/CD11b+ populations were found to be significantly reduced compared to the vehicle controls. While DFA, given 3 hours post-injury, improved BMS scores and white matter sparing to levels similar to that seen in mice treated immediately post-injury, no long-term benefit was seen when DFA was initiated at 8 hours post-injury. These collective data highlight L-selectin as a novel early and temporally specific initiator of pathogenesis, presumably through mechanisms underlying early recruitment of specific subsets of leukocytes, and support the repurposing of DFA for the treatment of SCI.

Disclosures: D.A. McCreedy: None. **C.J. Sontag:** None. **S.M. Lee:** None. **A.F. Martinez:** None. **T.M. Fandel:** None. **S.D. Rosen:** None. **L.J. Noble-Haeusslein:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.25/H24

Topic: C.10. Trauma

Support: Nutricia Research

Title: A combination of nutrients designed to enhance synapse formation and function improves functional outcome following spinal cord injury

Authors: *P. N. PALLIER[1], L. PODDIGHE[2], V. ZBARSKY[1], M. KOSTUSIAK[1], R. CHOUDHURY[1], T. HART[1], M. A. BURGUILLOS[1], O. MUSBAHI[1], M. GROENENDIJK[3], J. W. SIJBEN[3], M. C. DE WILDE[3], M. QUARTU[2], J. V. PRIESTLEY[1], A. T. MICHAEL-TITUS[1];
[1]Neurosci. and Trauma, Queen Mary Univ. of London, London, United Kingdom; [2]Dept. of Biomed. Sciences, Section of Cytomorphology, Univ. of Cagliari, Cagliari, Italy; [3]Nutricia Research, Nutricia Advanced Med. Nutr., Utrecht, Netherlands

Abstract: Spinal cord injury (SCI) leads to major neurological impairment, associated with significant tissue loss. Endogenous repair processes occur following SCI, but they are limited. Recent clinical trials in Alzheimer's disease have demonstrated the efficacy of Fortasyn® Connect (FC), a specific multinutrient combination that was designed to compensate for the loss of neuronal membranes and synapses in dementia patients, and that contains docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), choline, uridine monophosphate, phospholipids, folate, vitamins B6, B12, C, E and selenium. We tested if this multinutrient combination countered the tissue destruction occurring after SCI and supported regenerative processes, improving the neurological outcome. Adult rats received an injury induced by cord compression at thoracic level, and immediately after SCI they were fed daily with a control diet or a diet supplemented with different doses of the specific FC multinutrient combination (low dose FC, medium dose FC, or high dose FC) for 4 or 9 weeks. At 4 weeks, only 50% of rats that were fed the control diet were able to plantar place their paws, and only 2 rats had recovered gait coordination. In contrast, 6 out of 7 rats fed the diet with the high dose of FC had recovered a coordinated gait. Five of them showed a normal position of the paws and full recovery of toe clearance, and 2 of them showed a gait that was undistinguishable from that of uninjured rats. The BBB score was 17.1 ± 1.6 in this group, in comparison with the BBB score of 8.8 ± 1.3 in rats fed the control diet. This was accompanied by significant protection of oligodendrocytes and myelin in the injured tissue, a decreased microglial neuroinflammatory response, and an increase in pre- and postsynaptic markers. The medium dose of FC that did not show efficacy after 4 weeks of treatment led to improved motor score, increased neuronal and oligodendrocyte survival, decreased microglial activation, and better axonal preservation after 9 weeks of supplementation. These results suggest that a diet supplemented with this specific multinutrient combination has marked therapeutic potential in SCI.

Disclosures: P.N. Pallier: None. **L. Poddighe:** None. **V. Zbarsky:** None. **M. Kostusiak:** None. **R. Choudhury:** None. **T. Hart:** None. **M.A. Burguillos:** None. **O. Musbahi:** None. **M. Groenendijk:** None. **J.W. Sijben:** None. **M.C. de Wilde:** None. **M. Quartu:** None. **J.V. Priestley:** None. **A.T. Michael-Titus:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.26/H25

Topic: C.10. Trauma

Support: NRF-2013R1A1A2013440

Title: Effect of PPAR-γ agonist on inflammatory mediators in rats following spinal contusion

Authors: J. OH[1], G. PARK[1], Y. KIM[1], J. KIM[2], *Y. W. YOON[1];
[1]Korea Univ. Col. Med., Seoul, Korea, Republic of; [2]Korea Univ. Col. Hlth. Sci., Seoul, Korea, Republic of

Abstract: Lipid metabolism is important to the nervous system owing to the myelin sheath. After spinal cord injury, inflammatory mediators such as eicosanoids are released and they act as peroxisome proliferators that activate peroxisome proliferator activated receptors (PPARs), which play a key role in lipid metabolism and cellular differentiation. PPARs are classified into three isoforms (α, β/δ, γ) in the spinal cord. Above all, PPAR-γ is the most studied and its agonist, pioglitazone, is clinically used for the diabetes treatment. Previously, PPAR-γ shows as an anti-inflammation action. Thus, we investigated how the PPAR-γ agonist affects inflammatory mediators and motor function recovery in spinal contusive rats. Male Sprague-Dawley rats were anesthetized with ketamine/rompun mixture (1:4). Ten gram weight dropped from 12.5 mm height onto the spinal cord of the 10th thoracic vertebral level in rats after laminectomy using NYU device. We examined protein expression of PPAR-γ in the rostral, caudal, injured epicenter and L4-5 of the spinal cord at various times after injury (6, 12, 24h, 3d, 1, 3 and 5 weeks). Pioglitazone or vehicle (100% DMSO) was administered by intrathecal or intraperitoneal injection in the early phase and late phase after SCI. After drug administration, motor function was assessed using a BBB locomotor scale, and mRNA expression of inflammatory mediators was analyzed by real-time PCR. Protein expression changes of PPAR-γ was excessively increased from 6 hours and the increment was maintained for a long time after injury. That was the greatest in the epicenter and affected to the remote L4-5 spinal segments. In the early phase, intraperitoneally injected pioglitazone did not affect motor function compared with vehicle. Recovery of the joint movement was improved by Intrathecal injection of pioglitazone, however, no difference after that. In the late phase when PPAR-γ expression returned to basal level, intrathecal administration of pioglitazone restrained locomotion. After intrathecal administration of pioglitazone, protein expression of PPAR-γ and α was increased but not changed beta/delta in spinal contusive rats. On the contrary to previous reports, mRNA expression of iNOS, COX-2, IL-1B, IL-6 and chemoattractants was increased after that. Therefore, our results demonstrated that the PPAR-γ agonist may be positively help to induce the essential inflammatory mediators in the early phase, but may exacerbate motor recovery in the late phase after spinal cord injury.

Disclosures: J. Oh: None. **G. Park:** None. **Y. Kim:** None. **J. Kim:** None. **Y.W. Yoon:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.27/H26

Topic: C.10. Trauma

Support: Melo e Castro Santa Casa da Misericordia Award 2013

Title: Acute neuroprotection after spinal cord injury - going solo or in need of a friend

Authors: ***N. SILVA**, N. VASCONCELOS, E. GOMES, E. OLIVEIRA, C. SILVA, R. SILVA, R. LIMA, N. SOUSA, A. SALGADO;
Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Hlth. Sci., Braga, Portugal

Abstract: Damage to the spinal cord can result in irreversible impairments or complete loss of motor, autonomic and sensory functions. Systemically administered riluzole and magnesium chloride have been widely investigated as neuroprotective agents in animal models of spinal cord injury (SCI) and were found to promote both locomotor improvements and tissue sparing after SCI. Therefore, we aimed to investigate the neuroprotective efficacy of individual and combined administration of these drugs. An *in vivo* experiment was set using nineteen female Wistar-Han rats that underwent a thoracic spinal cord contusion (T8) using a weight drop method to induce severe injury. An hour after injury, animals were randomly distributed to receive: 1) riluzole (2.5 mg/kg,) 2) magnesium chloride (24.18 mg/kg) in a PEG formulation, 3) a combined treatment (riluzole and magnesium), or 4) saline. Subsequent treatments were given in 4 intraperitoneal injections (spaced 12 hours apart). The Basso, Beattie, and Bresnahan (BBB) locomotor rating scale, an activity box test, and a swimming test were used to evaluate behavioral recovery. Histological analysis of the spinal cords was performed to measure the extent and volume of the lesion, neuronal survival, inflammation, axonal preservation, seratonergic and glutamatergic fiber sparing, and myelin degeneration. Our results show that only the riluzole treatment significantly improved behavioral recovery, promoted tissue sparing, diminished lesion volume, increased seratonergic fiber sparing and axonal preservation in the caudal portion of the spinal cord. The combined treatment, although simultaneously targeting several excitotoxic-related mechanisms, did not further improve behavioral and histological outcome when compared with riluzole given alone.

Disclosures: **N. Silva:** None. **N. Vasconcelos:** None. **E. Gomes:** None. **E. Oliveira:** None. **C. Silva:** None. **R. Silva:** None. **R. Lima:** None. **N. Sousa:** None. **A. Salgado:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.28/H27

Topic: C.10. Trauma

Support: P20MD006988

Title: Functional recovery conferred by a prophylactic Omega-3 and Vitamin E-enriched diet and its potential link to the scavenger receptor/lipid transporter CD36 in spinal cord injury

Authors: *K. CORDERO, J. D. FIGUEROA, M. DE LEÓN;
Loma Linda Univ. Sch. of Med., Loma Linda, CA

Abstract: Spinal cord injury (SCI) is a major cause of disability in the US, devastating the lives of more than 12,000 young adults annually. Although SCI may prove difficult to prevent in most instances, there is a need to further investigate factors affecting susceptibility to damage in the event of injury. In ongoing studies, we have shown that the metabolism of fatty acids is markedly deregulated acutely after SCI, determining the extent of functional recovery after such insult. Emerging observations from our lab show that omega-3 polyunsaturated fatty acids (O3PUFAs) and vitamin E are major modulators of repair mechanisms following SCI in rodents. However, the molecular mediators coupling these beneficial fatty acids to functional recovery after SCI remain understudied. The fatty acid translocase membrane cluster of differentiation 36 (FAT/CD36) is a B class scavenger receptor highly expressed in models of neurological injury and implicated in the uptake and signaling of fatty acids and vitamin E. FAT/CD36 have been shown to be both beneficial and detrimental after injury. Our preliminary evidence suggests that the lipid microenvironment may determine the role of FAT/CD36 after injury. Our objectives are to 1) characterize the spatiotemporal expression and functional roles of CD36 after SCI and 2) determine how if FAT/CD36 contributes to Vitamin E-mediated protection. Our central hypothesis states that FAT/CD36 is acutely upregulated following SCI and its blockade impairs the protective effects of vitamin E and possibly other hydrophobic molecules. This hypothesis is based on: 1) FAT/CD36 ability of transporting hydrophobic molecules such as O3PUFAs and Vitamin E 2) our studies showing that O3PUFAs are neuroprotective after SCI. Our rationale is that, knowledge of how O3PUFAs and vitamin E modulate protection after SCI will provide the foundation for the development of interventions aimed at restoring function. This is expected to help to reduce the disease burden by identifying novel therapeutic targets. We analyzed locomotion recovery, H-reflex depression, bladder recovery, and the temporo-spatial expression of CD36 after contusive injury to the rat spinal cord using behavioral and electrophysiogical studies, Credé's maneuver, immunoblotting and double-labeling immunofluorescent experiments. Our data demonstrates that Vitamin E and O3PUFAs accelerate locomotor and bladder recovery, improves H-reflex depression, and that acute SCI results in a marked upregulation of CD36. Our next step is to determine a possible mechanism through which Vitamin E is improving functional recovery and find if there is a link between Vitamin E protection and CD36 after SCI.

Disclosures: **K. Cordero:** None. **J.D. Figueroa:** None. **M. De León:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.29/H28

Topic: C.10. Trauma

Support: NIH/NINDS NS049545 & NS30291

DOD W81XWH-05-1-0061

Title: Ephb3 receptors function as dependence receptors to mediate oligodendrocyte cell death following contusive spinal cord injury

Authors: *Y. E. TSENKINA[1], J. RICARD[2], E. RUNKO[1], M. M. QUIALA-ACOSTA[1], J. MIER[1], D. J. LIEBL[1];
[1]Neurosurg., Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Dept. of Biol., Drexel Univ., Philadelphia, PA

Abstract: Spinal cord injury (SCI) leads to functional impairment resulting from regional cell death. SCI-induced apoptosis persists for days to weeks after the initial trauma, where the myelin-producing oligodendrocytes (OLs) are highly susceptible. OL loss underlies progressive demyelination and axonal degeneration contributing to motor disability. Therefore, it is important to examine the mechanisms leading to OL death in the injured spinal cord. Previously, we identified new pro-apoptotic receptors, belonging to the dependence receptors family, that activate terminal caspases in the absence of their cognate ligand(s). Dependence receptors fulfill dual functions depending on their ligand availability. During normal development and tissue homeostasis, they interact with their ligand(s) to promote cell survival, migration and differentiation. However, when deprived from their ligand(s), they can trigger and/or amplify caspase-mediated apoptosis. Ephrins and Eph receptors are membrane-bound proteins that require cell-cell interactions and are known regulators of axon path finding, cell proliferation, migration and synaptic plasticity. In the adult central nervous system (CNS), EphB3 receptors regulate neural progenitors and neuronal survival following traumatic brain injury, supporting a dependence receptor role for EphB3. These studies also revealed that EphB3 could have a pro-apoptotic role enhancing the survival of multiple cell types in the injured CNS. In the present study, we demonstrate that EphB3 receptors mediate OL cell death in the injured spinal cord through dependence receptor mechanism. OLs in the adult spinal cord express EphB3 as well as other members of the Eph receptor family. SCI is associated with tissue damage, cellular loss and disturbances in EphB3-ephrinB3 protein balance acutely after the insult creating an environment for a dependence receptor-mediated cell death to occur. Ablation of EphB3 promotes OL survival associated with increased expression of myelin basic protein and improved locomotor function in mice after SCI. Furthermore, chronic infusion of ephrinB3 to the spinal cord after injury also promotes OL survival. Our *in vivo* findings are supported by *in vitro* studies showing that ephrinB3 administration promotes the survival of both oligodendroglial progenitor cells as well as mature OLs cultured under pro-apoptotic conditions. Our study demonstrates a novel dependence receptor role of EphB3 in OL cell death after SCI, and supports further development of ephrinB3-based therapies to promote recovery.

Disclosures: **Y.E. Tsenkina:** None. **J. Ricard:** None. **E. Runko:** None. **M.M. Quiala-Acosta:** None. **J. Mier:** None. **D.J. Liebl:** None.

Poster

310. Spinal Cord Injury: Animal Models and Human Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 310.30/H29

Topic: C.10. Trauma

Title: De-novo myelination of grafted neural progenitor cells in the adult cns

Authors: *M. A. HUNT[1], P. LU[2,3], M. H. TUSZYNSKI[2,3];
[1]Biomed. Sci., [2]Neurosci., Univ. of California San Diego, LA Jolla, CA; [3]Veterans Affairs Med. Ctr., San Diego, CA

Abstract: We previously reported that neural progenitor cells (NPCs) grafted into sites of spinal cord injury (SCI) exhibit extensive axonal outgrowth and formation of new synaptic connections with host neurons, forming novel functional neural relays across lesion sites. In the present study we performed a detailed characterization of axons emerging from NPC grafts. Rat embryonic spinal cord-derived NPCsderived NPCs, constitutively expressing EGFP, were grafted into adult C5 spinal cord hemisections. Three months after grafting, we examined graft-derived axonal diameter and myelination using transmission electron microscopy. In total, 104 graft-derived axons were characterized. Axon diameter ranged from 0.15 to 1.70 μm, and 23% of graft-derived axons were myelinated. The average diameter of myelinated axons (0.72 ± 0.3μm) was significantly larger than that of non-myelinated axons (0.61 ± 0.2μm, $p<0.05$). Notably, the G-ratio of myelinated graft-derived axons was comparable to that of developmentally myelinated axons (0.77 ± 0.05 μm). 67 % of graft-derived axons were in direct contact with host myelin, suggesting that myelin does not repel axons extending from implants of early stage neurons. Acknowledgements: Veterans Administration and the Adelson Medical Research Foundation

Disclosures: M.A. Hunt: None. **P. Lu:** None. **M.H. Tuszynski:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.01/H30

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R21 MH091774

Title: Attentional effects on the auditory steady-state response in schizophrenia-related disorders

Authors: *L. MORAVEC[1], J. M. HOWELL[2], D. R. WESTFALL[3], D. EWBANK[2], W. P. HETRICK[2], A. R. BOLBECKER[2], A. BREIER[4], B. O'DONNELL[2];
[1]Psychological and Brain Sci., Bloomington, IN; [2]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [3]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [4]Dept. of Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Auditory steady state response (ASSR) measures of power and phase synchronization have been reported to be reduced in schizophrenia, most consistently to 40 Hz stimulation. It is possible that a task requiring selective attention to a 40 Hz stimulus might reverse this deficit. In order to test this hypothesis, click trains were used to generate ASSRs at different frequencies of stimulation in an auditory discrimination task. Sixty four patients with a psychotic disorder (schizophrenia, schizo-affective, schizophreniform, or psychotic NOS) and 126 healthy control subjects were tested. The auditory discrimination paradigm used three different stimuli: a 20 Hz click train, a 40 Hz click train, and a distractor tone. Each stimulus was 1000 ms in duration presented at 90 dB SPL with 500 ms inter-stimulus interval. Two conditions were used, each with 400 stimuli. In one condition, the 40 Hz click train was the target; in the second condition, the 20 Hz click train was the target. The subject pressed a button to respond to target stimuli. Within a condition, stimulus probabilities were: target click train (p=.15), non-target click train (p=.70) and distractor tone (p=.15). A 32 channel electroencephalogram (EEG) was recorded from each subject with a sampling rate of 1000 Hz (bandpass:0.1 to 100 Hz). The EEG data was sampled at 1000Hz/channel and filtered from 0.1 to 100 Hz. ASSRs were obtained after averaging EEG across trials for the 20 and 40 Hz click train responses in each condition and applying a fast-Fourier transform to measure power at the stimulus frequency during the stimulus period. Independent sample t-tests showed that 40 Hz power was reduced for both the target and non-target 40 Hz click trains; while 20 Hz power did not differ between groups in either condition. Thus, the 40 Hz deficit observed in schizophrenia-related psychotic disorders appears regardless of the task relevance of a stimulus. Conversely, 20 Hz stimuli are not associated with an disturbed ASSR, even at high levels of attentional demand. These data provide further support for a selective gamma frequency range deficit in the auditory modality in schizophrenia.

Disclosures: **L. Moravec:** None. **J.M. Howell:** None. **D.R. Westfall:** None. **D. Ewbank:** None. **W.P. Hetrick:** None. **A.R. Bolbecker:** None. **A. Breier:** None. **B. O'Donnell:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.02/H31

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NARSAD Young Investigator Award

NIMH P50 MH084053

NIMH P50 MH103204

NIMH K08 MH080329

Title: Prior cannabis abuse exacerbates impairments in action monitoring in first episode psychosis

Authors: *C. P. WALKER[1], S. BANGALORE[2], E. BELCHER[3], M. CARL[4], R. ZENISEK[5], R. Y. CHO[6];
[1]Psychiatry, Univ. of Texas Hlth. Sci. Ctr. At Houst, Houston, TX; [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Translational Biomed. Sci., Univ. of Rochester, Rochester, NY; [4]Dept. of Psychology, Univ. of Tennessee, Knoxville, TN; [5]Dept. of Psychology, Univ. of Nevada, Las Vegas, Las Vegas, NV; [6]Psychiatry, Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Mounting evidence suggests a close relationship between the pathophysiologic mechanisms underlying psychosis and cannabis abuse. This association may be unsurprising, given the dense expression of the cannabinoid CB1 receptors in regions associated with executive functions and action monitoring which are known to be impaired in psychotic illness. One of the critical impairments in psychosis involves the anterior cingulate cortex (ACC) which supports action monitoring processes. However, the impacts of prior cannabis usage on cognitive control and ACC function in psychosis are as yet unexplored. To that end, we recruited 76 participants (24F, M_{age}=22) for an EEG study of the Stroop task. Participants were 34 psychiatrically normal controls, half of whom reported current cannabis abuse and 42 first-episode, med-naive psychosis patients, half of whom were comorbid for cannabis abuse. High-density EEG data were collected, and correct and error response-locked event-related potentials (ERPs) were submitted to a three-stage analysis pipeline using a combination of principal component analysis, independent component (IC) analysis, and permutation testing to reduce noise and data dimensionality. We identified three ICs corresponding to the expected spatial and temporal patterns of traditional ERP analyses of the error-related negativity (ERN; 0-100 ms, frontal distribution) and error-positivity (Pe; 200-400 ms, posterior distribution) which colocalize to generators in the ACC. Behaviorally, cannabis abusers (p=0.009) and psychosis patients (p=0.045) responded slower than healthy non-users. These effects were found to be additive, with no effects found for accuracy. ERP analyses revealed an anterior ERN IC demonstrating reduced amplitudes for cannabis abusers only (p=0.025) irrespective of psychosis diagnosis status, while a posterior Pe IC revealed a cannabis by psychosis interaction (p=0.041) where cannabis abusing patients were found to have a smaller Pe compared to all other groups. This pattern suggests error detection processes are relatively conserved in first-episode psychosis except in the context of comorbid cannabis abuse which then resembles impairments more typical of chronic patients. To our knowledge, this is the first investigation of the effects of cannabis abuse on psychosis and action monitoring, highlighting the link between cannabis abuse and early exacerbation of cognitive deficits in psychotic illnesses.

Disclosures: **C.P. Walker:** None. **S. Bangalore:** None. **E. Belcher:** None. **M. Carl:** None. **R. Zenisek:** None. **R.Y. Cho:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.03/H32

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH R01: 5-R01-MH058251-06-10

Conte: 5-P50-MH064065-07-10

NARSAD Independent Investigator Award to AB

Foundation of Hope Award to AB

Title: Converging evidence of aberrant neural synchrony in schizophrenia and relatives

Authors: *E. H. ANDERSEN[1], A. M. CAMPBELL[2], S. E. SCHIPUL[2], S. E. SCHIPUL[2], A. BELGER[2];
[1]Psychiatry, Univ. of North Carolina, Chapel Hill, NC; [2]Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Schizophrenia (SCZ) is characterized by disruptions in both affective and cognitive processes. The integration of these processing domains relies on effective neural communication, which can be indexed by EEG neural synchrony measures of evoked power (EP) and phase-locking factor (PLF) in the time-frequency domain in response to events. In the current study, we examine neural synchrony in two paradigms that recruit the interplay of cognitive and affective systems, and how neural synchrony measures relate across tasks. Participants were recent onset SCZ patients (n=15), familial high-risk (HR, n=14) participants, and matched healthy controls (CON, n=17). The Emotional Oddball (EO) task was a visual oddball with emotional distractors. The Emotional Working Memory (WM) task was a 1-back task with positive, neutral, or negative emotional stimuli. Results EO: We found reduced EP and PLF in the delta band (1-4Hz) to targets in SCZ ($p<.05$) and HR ($p<.05$) relative to CON, reflecting impaired directed attention. Reduced EP ($p<.05$) and PLF ($p<.05$) in the theta band (4-8Hz) to aversive distractors was found in SCZ relative to CON, indicating aberrant task-irrelevant emotional processing. HR participants showed a unique enhancement of EP in beta (16-30Hz) to aversive distractors ($p<.05$), suggesting heightened sustained emotional processing. Emotional WM: A significant Group (CON, HR, SCZ) X Condition (positive, neutral, negative) interaction ($F(4, 74)=3.77$, $p<.01$) revealed elevated EP in theta in response to aversive stimuli for HR compared to CON ($p<.05$) and SCZ ($p=.01$), reflecting increased WM processing. Relating synchrony across tasks,

we found that sustained affective processing (beta EP) in the EO task related to inhibition (delta EP) necessary to maintain task goals ($r=.48$, $p<.05$) and to task-relevant aversive target theta EP ($r=.54$, $p<.001$) in the Emotional WM across groups. HR alone showed a correlation between affective processing to aversive stimuli (beta EP) and WM load-related theta EP for aversive targets (CON: $r=-.26$, $p=.39$; HR: $r=.61$, $p<.05$; z-diff=2.42, $p<.01$). This work suggests that crucial neural connectivity underlies the integration of affective and cognitive processing across both task-irrelevant and task-relevant emotional information. These connections show disruptions (reflected in neural synchrony) in both SCZ and HR, which may affect the integration or inhibition of emotional information within cognitive tasks. Future directions: fMRI data was also collected and analyzed for these paradigms and will provide further understanding of the neural circuitry supporting the dynamics between cognitive and affective systems.

Disclosures: E.H. Andersen: None. **A.M. Campbell:** None. **S.E. Schipul:** None. **S.E. Schipul:** None. **A. Belger:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.04/H33

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01-MH092638

Title: Spindle deficits, cognitive impairment and real-life functioning in schizophrenia

Authors: R. A. FOWLER[1,2], *D. CORRELL[1,2], B. BARAN[1,2], C. DEMANUELE[1,2], T. VUPER[1,2], B. SEICOL[3], C. CALLAHAN[3], E. PARR[3], R. STICKGOLD[3], D. MANOACH[1,2]; [1]Psychiatry, Massachusetts Gen. Hosp. (MGH), Charlestown, MA; [2]Athinoula A. Martinos Ctr. for Biomed. Imaging, Charlestown, MA; [3]Dept. of Psychiatry, Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Sleep spindles correlate with human intelligence and are thought to mediate sleep-dependent memory consolidation. Schizophrenia (SZ) patients have markedly reduced sleep-dependent consolidation of motor procedural memory that correlates with reduced sleep spindle density. We investigated whether decreased sleep spindle density also correlates with worse performance on cross-sectional cognitive measures and real-life functioning in SZ. In a baseline visit, 23 SZ patients and 27 demographically-matched healthy controls were administered the MATRICS Consensus Cognitive Battery (MCCB) and the Wechsler Abbreviated Scale of Intelligence (WASI). Only patients also completed the UCSD Performance-based Skills Assessment (UPSA) to assess real-life function. A week later, participants were monitored with inpatient polysomnography for two nights. They learned a motor sequence task (MST) on the

second night and were tested the following morning. Sleep spindles during stage 2 sleep (N2) were identified using a wavelet spindle detector. Spindle density was calculated as the number of spindles per minute of artifact-free N2 sleep and averaged across the two nights. SZ participants had significantly lower IQ estimates on the WASI and worse performance on the MCCB (composite score). Sleep data were available for a subset of participants (12 patients, 12 controls). Patients showed a non-significant reduction in spindle density compared to controls (2.12±.44 vs. 2.34±.48: t(22)=1.13, p=.27). Spindle density inversely correlated with UPSA scores (r=-.60, p=.04) and verbal IQ in patients only (trend, r=-.54, p=.07). These correlations are not significant with correction for multiple comparisons. There was no significant correlation between spindle density and MCCB scores in either group. In line with our previous results, we observed a positive trend level correlation of spindle density with overnight improvement on the MST (r=.49, p=.13). Contrary to our hypotheses, real life functioning and higher verbal IQ correlated with decreased spindle density. However, we replicated previous findings that spindle deficits in SZ correlate with motor procedural memory consolidation on the MST. These findings may reflect that sleep-dependent memory consolidation is more proximally related to spindle density than cross-sectional cognitive measures or functional outcome. We are presently increasing the sample and analyzing the remaining sleep data to better understand the relations between sleep spindles, cognitive measures and functional outcome.

Disclosures: **R.A. Fowler:** None. **D. Correll:** None. **B. Baran:** None. **C. Demanuele:** None. **T. Vuper:** None. **B. Seicol:** None. **C. Callahan:** None. **E. Parr:** None. **R. Stickgold:** None. **D. Manoach:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.05/H34

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH R01MH094358

Title: Hallucinations and immunoreactivity: IL-6 is elevated in subjects who experience 'Voices Conversing'

Authors: C. ROSEN[1], K. A. CHASE[1,2], B. FEINER[1], J. MELBOURNE[1], H. GIN[1], *R. P. SHARMA[1,3];
[1]Dept Psychiatry, Univ. of Illinois at Chicago, Chicago, IL; [2]Dept. of Human Genet., Univ. of Chicago, Chicago, IL; [3]Jesse Brown Veterans Affairs Med. Ctr., Chicago, IL

Abstract: Background: Within a spectrum model of psychosis, utilizing a finely grained map of hallucinatory experiences allows for the integration of an underlying symptom dimension to

possible biological or pharmacological measures. Thus, the details of a hallucinatory experience such as ‘voices conversing’ may be better understood by an association with a biological variable such as an immunoreactive molecule. This type of analysis is relatively unexplored. Methods: Seventy-four participants with present-state psychosis with AVHs were recruited from the University of Illinois at Chicago. Inclusion criteria for the study included persons between the ages of 21 - 60 who were currently experience auditory verbal hallucinations. The results of this study constitute the findings of an investigation that explores quantitative and qualitative methodologies of the Schneiderian classification of auditory hallucinations, specifically of voices conversing. Participants were categorized as experiencing ‘voices conversing’ or ‘voices not conversing,’ and symptom differences were compared on the PANSS subscales. Levels of IL-6 mRNA were examined in peripheral blood mononuclear cells (PBMC) from these subjects through real-time RT-PCR. IL-6 mRNA values were normalized to GAPDH and β-Actin, and computed using a geometric mean. Results: When comparing these two groups, we found that participants who experienced voices conversing also demonstrated increased positive symptoms, cognitive disorganization, depressive symptoms and thought disturbances than those who did not experience voices conversing. Additionally, IL-6 mRNA levels were significantly elevated in those participants who did experience voices conversing when compared to those who did not experience voices conversing. Discussion: AVHs are among the most experientially complex transdiagnostic symptoms; thus research that explores potential clinical and biological associations can have significant translational and nosological implications. In this exploratory analysis, we demonstrate that immunoreactivity, a widely supported abnormality in psychotic disorders, is also enhanced in Schneiderian based symptomology of voices conversing, which may indicate a symptom specificity that extends beyond diagnostic characterization.

Disclosures: **C. Rosen:** None. **K.A. Chase:** None. **B. Feiner:** None. **J. Melbourne:** None. **H. Gin:** None. **R.P. Sharma:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.06/H35

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: FONDECYT N° 11140464

CONICYT-PCHA, Doctorado Nacional, 2014-21141115

Biomedical Neuroscience Institute, BNI

Title: Magnocelullar deficits in high-risk psychosis syndrome: an ERP study

Authors: *S. A. CORRAL[1], B. ABURTO[1], R. CASTILLO[1], R. MAYOL[1], A. MATURANA[1], V. DE ANGEL[1], D. GONZALEZ[1], H. SILVA[1], A. MARTINEZ[2], P. A. GASPAR[1];
[1]Univ. of Chile, Santiago, Chile; [2]Columbia Univ., New York, NY

Abstract: Background: Cognitive deficits found in schizophrenia (SZ) have been widely studied and may be mainly explained by changes in multimodal cortical areas. However, recent evidence suggests that the deficit could be begin at a sensory processing level. This can be measured by visual event-related potentials (ERP) such as the P1/N1 complex, mainly in the magnocellular pathway. The aim of this study is to determine whether there is a deficit in visual attentional processing in UHR subjects and evaluate potential alterations in the magno and parvocellular pathways. Methods: In this study (N = 6) healthy controls (HC) were evaluated and (N = 6) patients with UHR syndrome as diagnosed by the Structured Interview for Prodromal Symptoms (SIPS). Both groups were presented with visual attention stimuli on a screen where high and low spatial frequency (SF) gabors were observed. These were designed to evaluate the performance of the parvocellular and magnocellular pathways respectively. During the execution of this task, electrical activity was recorded using a Biosemi 64-channel electroencephalogram (EEG). The average evoked activity by an analysis of event related potentials (ERP) was then obtained. Results: In the low SF condition, the amplitude of the P1/N1 complex was altered in the UHR group compared to HC. Additionally, the UHR group had a significantly lower number of correct responses in low SF stimuli in the task. Conclusions: Amplitude changes in the P1/N1 complex in the magnocellular pathway in patients with SZ have been consistently found in previous literature. However there is little evidence of this in an UHR population. The abnormality in the amplitude of the P1/N1 complex observed in people with UHR may be important to understand visual sensation alterations in this at risk population.

Disclosures: S.A. Corral: None. **B. Aburto:** None. **R. castillo:** None. **R. mayol:** None. **A. maturana:** None. **V. de angel:** None. **D. gonzalez:** None. **H. silva:** None. **A. martinez:** None. **P.A. gaspar:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.07/H36

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: JSPS 15K19735

Title: Gamma band auditory steady-state responses in first episode psychosis and chronic schizophrenia

Authors: *N. ORIBE[1,2], H. KUGA[2], I. NAKAMURA[1], Y. HIRANO[1], T. ONITSUKA[1], T. UENO[2];
[1]Kyushu Univ., Fukuoka, Japan; [2]Natl. Hosp. Organization Hizen Psychiatric Ctr., Yoshinogari, Japan

Abstract: Background Gamma band auditory steady state response (ASSR) have been reported to be abnormal and considered to be a robust biomarker in chronic schizophrenia patients. However, whether ASSR is compromised in the early course of the illness or not is yet to be determined since there have been only a few reports regarding with the ASSR in first episode schizophrenia. In addition, none of the previous studies employed higher frequency stimuli than 40Hz. Methods We measured ASSR responses in chronic schizophrenia patients (SZ, N=26), first episode psychosis patients (FEP, N=6) as well as healthy control subjects (HC, N=35) while presenting click trains varying in rate of stimulation (20, 30, 40 and 80 Hz). EEG-evoked power and phase locking were obtained in response to each stimulation frequency. Results SZ showed significant reduction in phase locking to the 40Hz stimuli compared with HC while FEP showed intact responses both in phase locking and evoked power to the 40Hz stimuli. There were no significant group differences in responses to other frequency bands. Conclusions These results suggest that neural circuits abnormalities indexed by the ASSR are differentially altered in the course of the illness.

Disclosures: N. Oribe: None. **H. Kuga:** None. **I. Nakamura:** None. **Y. Hirano:** None. **T. Onitsuka:** None. **T. Ueno:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.08/H37

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: RO1MH091348

Title: Chondroitin Sulfate Proteoglycan expression in the human olfactory system: implications for the pathophysiology of schizophrenia

Authors: *F. J. HAMATI[1], A. BOYER-BOITEAU[1], H. PANTAZOPOULOS[1], S. BERRETTA[1,2];
[1]Translational Neurosci. Lab., McLean Hosp., Belmont, MA; [2]Dept. of Psychiatry, Harvard Med. Sch., Boston, MA

Abstract: Background: Chondroitin sulfate proteoglycans (CSPGs), one of the main components of the brain extracellular matrix, were found to be altered in the medial temporal lobe, prefrontal cortex and olfactory epithelium (OE) of people with schizophrenia (SZ). These molecules are

robustly expressed in the olfactory system, where they regulate neuronal differentiation and axon guidance. Interestingly, odor identification deficits have been observed in patients with SZ and first-degree relatives. Together, these observations suggest that a disruption of CSPG expression in the olfactory system may contribute to the olfactory deficits observed in SZ. As a first step toward testing this hypothesis, we are investigating the pattern of CSPG expression in the olfactory mucosa (OM) and bulb (OB) of healthy individuals. More precisely, we are testing the hypothesis that distinct CSPGs surround olfactory axon bundles, forming channels that guide them through the OM to their odor-specific glomeruli within the OB. Methods: Dual and triple antigen immunofluorescence was used on OM and OB sections from healthy human subjects to label the axons of olfactory receptor neurons (ORN), and investigate their relationship with CSPGs, as labeled with the lectin, wisteria floribunda agglutinin (WFA), or Aggrecan and 6-sulfated (CS56) CSPG-immunodetection. Results: In the OM, CSPGs form complex, channel-like structures that tightly surround ORN axon bundles. Distinct channel-like structures were labeled by different CSPGs with no detectable overlap, suggesting CSPG-specific segregation of ORN axon bundles. In the OB, CSPG-labeling surrounds incoming axonal bundles along the superficial olfactory nerve layer in addition to their corresponding glomeruli. However, consistent with the pattern in the OM, distinct CSPGs showed preferential and non-overlapping distribution patterns, for instance, CS56-IR was found only superficially, exclusively surrounding ORN axon bundles but not the glomeruli, while WFA-IR showed opposing medial expression patterns. Conclusion: Our results show that non-overlapping CSPGs form channel-like structures that surround ORN axon bundles. Although preliminary, our results suggest that, in the human olfactory system, distinct CSPGs guide these ORN axon bundles to their corresponding odor-specific glomeruli. If so, disruptions of CSPG expression in the olfactory system of patients with SZ may contribute to the pathophysiology underlying odor identification deficits observed in this disorder. These results provide useful insights into the role CSPGs play in regulating axon guidance during brain development.

Disclosures: F.J. Hamati: None. **A. Boyer-Boiteau:** None. **H. Pantazopoulos:** None. **S. Berretta:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.09/H38

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: FONDECYT N° 11140464

CONICYT-PCHA, Doctorado Nacional, 2015-21150063

Biomedical Neuroscience Institute

Title: Abnormalities in P300 reveal impaired working memory processing in subjects at high-risk of psychosis

Authors: R. I. CASTILLO[1,2], R. MAYOL[3], S. A. CORRAL[2], B. ABURTO[2], V. DE ANGEL[1], H. SILVA[1,4], D. GONZALEZ[2], *P. A. GASPAR[5,1,4,2];
[1]Psychiatry, Univ. of Chile Clin. Hosp., Santiago, Chile; [2]Translational Psychiatry Lab., Fac. of Medicine, Univ. of Chile, Santiago, Chile; [3]Univ. of Chile, Faculty of Medicine, Chile; [4]Biomed. Neurosci. Inst., Santiago, Chile; [5]Lifes Sciences., Nathan Kline Inst., Orangeburg, NY

Abstract: Background: Working memory (WM) abnormalities represent a core cognitive dysfunction in Schizophrenia (SZ). The decrease of the P300 visual evoked-potential (VEP) component is considered a physiological phenomenon when increasing WM load. Abnormalities of P300 amplitude have been classically associated to neurophysiological abnormalities of WM processing in SZ. However, little is known about these abnormalities in high-risk state psychosis (HR) population. Methods: To further investigate this topic, we performed a 64-channel electroencephalogram (EEG) while executing a Sternberg WM task in 6 HR subjects and in 6 matched healthy controls (HC). Then we did a VEP analysis in order to study the neurophysiological mechanisms underlying the WM cognitive processing in this population. We also compared these data to WM behavioral scores in all subjects. Results: Controls showed an inverse correlation between P300 amplitude and WM load. In contrast, HR subjects showed a lower amplitude in all conditions. Lower WM behavioral scores were also correlated with P300 amplitude abnormalities in HR subjects. Conclusions: These VEP P300 findings are consistent with previous research in SZ patients. Lower WM behavioral scores and their correlation with P300 abnormalities might explain in part the social difficulties seen in these subjects.

Disclosures: R.I. Castillo: None. **R. Mayol:** None. **S.A. Corral:** None. **B. Aburto:** None. **V. de Angel:** None. **H. Silva:** None. **D. Gonzalez:** None. **P.A. Gaspar:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.10/H39

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: OMHF Studentship

Title: The predictive power of early treatment response in first-onset psychosis: a randomized controlled trial of haloperidol versus olanzapine

Authors: *S. RASMUSSEN, P. I. ROSEBUSH, M. F. MAZUREK;
McMaster Univ., Hamilton, ON, Canada

Abstract: Background: Early response to antipsychotic treatment has emerged as a powerful predictor of treatment outcome, but there is some evidence that the predictive value of early response is poor for patients treated with olanzapine. Additionally, it is unclear whether the predictive value of early response persists throughout long-term treatment. Methods: We prospectively studied a cohort of antipsychotic-naive, first-episode psychosis patients randomized to treatment with haloperidol or olanzapine. All patients were assessed at baseline and twice weekly thereafter using the Brief Psychiatric Rating Scale (BPRS), Hamilton Depression Rating Scale (HAM-D), Hamilton Anxiety Rating Scale (HAM-A), and Young Mania Rating Scale (YMRS). Regression analyses were used to determine whether improvements on these measures at week 2 predicted improvements at discharge and at long-term follow-up. Results: 93 patients were assessed throughout hospitalization and at discharge. Length of hospital stay and overall treatment response were similar between treatment groups. In the haloperidol group, week 2 improvement was associated with improvement at discharge for BPRS total ($p<.001$), BPRS psychotic symptom subscale ($p=.010$), HAM-D ($p<.001$), HAM-A ($p=.019$), and YMRS scores ($p=.008$). In the olanzapine group, by contrast, week 2 improvement predicted improvement at discharge only for HAM-D scores ($p=.034$). 64 patients were assessed at follow-up between 14 and 39 months after their initial admission (mean=25 months). In this cohort, early response to haloperidol predicted long-term changes in BPRS total ($p=.001$), BPRS psychotic symptom subscale ($p<.001$), HAM-D ($p=.050$), and YMRS scores ($p=.049$), but not HAM-A scores ($p=.263$). Early response to olanzapine did not predict long-term outcomes on any measure. Conclusion: Early response after 2 weeks of treatment with haloperidol was predictive of improvement at discharge and at long-term follow-up 1-3 years after the initial admission. Early response to olanzapine, on the other hand, did not predict treatment outcome at either time point. These results demonstrate for haloperidol the long-term prognostic value of assessing early response to antipsychotic treatment, but suggest that, in the case of olanzapine, a more prolonged therapeutic trial may be required to assess long-term treatment efficacy.

Disclosures: S. Rasmussen: None. **P.I. Rosebush:** None. **M.F. Mazurek:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.11/H40

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: JSPS KAKENHI Grant Number 15K21648

Title: Study for the effect of carbonyl stress makers on cognitive impairment of schizophrenia

Authors: *A. KOBORI[1], S. HATAKEYAMA[1], Y. HORIUCHI[1], K. TORIUMI[1], M. MIYASHITA[2], M. ITOKAWA[1], H. ARAI[3], M. ARAI[1];

[1]Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Shinsyu Univ. Sch. of Med., Nagano, Japan; [3]Juntendo Univ. Grad. Sch. of Med., Tokyo, Japan

Abstract: We investigated association between cognitive impairment and carbonyl stress with high plasma pentosidine and low serum vitamin B6 (pyridoxal) in schizophrenia. We divided 44 subjects with schizophrenia into 4 groups by levels of carbonyl stress markers (Group I: normal pentosidine & pyridoxal, Group II: normal pentosidine & low pyridoxal, Group III: high pentosidine & normal pyridoxal, Group IV: high pentosidine & low pyridoxal), and assessed the symptom severity by the Manchester Scale Japanese version and the cognitive function by Wechsler Adult Intelligence Scale 3rd (WAIS-III) and Wisconsin card sorting test (WCST). Data showed that schizophrenics with carbonyl stress (Group IV) had lower score of "picture completion" on WAIS, suggesting their impairments of long-term memory with visual attention in the trial. Performance Intelligence Quotient (PIQ) is a score resulting from a test that assesses capacity in dealing with non-verbal skills. "Picture completion" and "Picture arrangement" subtests of PIQ which is a non-verbal ability to perceive visual details. Our preliminary data suggest that schizophrenic patients with carbonyl stress might be associated with impairment of eye-hand coordination via visual working memory as visual attention.

Disclosures: A. Kobori: None. **S. Hatakeyama:** None. **Y. Horiuchi:** None. **K. Toriumi:** None. **M. Miyashita:** None. **M. Itokawa:** None. **H. Arai:** None. **M. Arai:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.12/H41

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Detection of brain correlates of Schizophrenia deficits with a novel electroencephalography mobile system

Authors: *R. GIL-DA-COSTA[1,2], R. FUNG[2], M. CASWELL[2], T. P. COLEMAN[3], G. LIGHT[4]; [1]Systems Neurobio. Labs., Salk Inst. for Biol. Studies, LA JOLLA, CA; [2]Neuroverse, Inc., San Diego, CA; [3]Depart. Bioengineering, [4]Depart. Psychiatry, UCSD, San Diego, CA

Abstract: According to the National Institute of Mental Health, neuropsychiatric disorders will continue to affect approximately 46.4 percent of the U.S. adult population, with approximately 22.3 percent of these cases classified as "severe" (Statistics: Any Disorder Among Adults, National Institute of Mental Health). However, unlike electromyography biosensors for cardiac monitoring or diabetes glucose sensors, where a wide range of efficient commercial solutions are available, there are no adequate general public technological solutions for brain monitoring and evaluation. In a society currently affected by a wide range of mental disorders that impose

exponentially growing heavy penalties at both the individual and societal levels, the need for such systems is paramount. Currently available cognitive monitoring solutions that can assist in early detection, diagnosis and treatment efficacy are cumbersome and ill-suited for regular use, and impossible for use at home by patients and caregivers. This leads to an almost complete lack of use by clinicians and the impossibility of individual regular monitoring, which is key in today's prevention and understanding of mental illnesses. Here, we present a case-study with schizophrenia patients and healthy subjects, where we used a novel mobile forehead electroencephalographic (EEG) sensing system, with a software application for testing and analysis in mobile platforms (e.g. smartphones and tablets). We compared modulations of brain function associated with well-studied brain markers in healthy individuals, and correlated with deficits in schizophrenia. Namely, three event-related brain potentials (the mismatch negativity (MMN), P3a and reorienting negativity (RON)), recorded using this novel mobile system in 60 subjects (30 schizophrenia patients and 30 healthy controls) during an auditory oddball paradigm task. Our results show significantly reduced amplitudes for all three ERPs in schizophrenia patients, as compared to both young and age-matched healthy subjects. This findings are consistent with previous reports associating these ERP modulations to deficits in cognitive updating and attention orientation in schizophrenia. Thus, this study presents evidence of the outstanding efficacy of a novel user-friendly mobile system that can open new avenues for much needed large-scale applications in neuropsychiatric deficit detection and evaluation, clinical outcome predictability and drug efficacy evaluation.

Disclosures: **R. Gil-Da-Costa:** A. Employment/Salary (full or part-time):; Neuroverse, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuroverse, Inc.. F. Consulting Fees (e.g., advisory boards); Neuroverse, Inc. **R. Fung:** A. Employment/Salary (full or part-time):; Neuroverse, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuroverse, Inc. **M. Caswell:** A. Employment/Salary (full or part-time):; Neuroverse, Inc. **T.P. Coleman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuroverse, Inc. **G. Light:** F. Consulting Fees (e.g., advisory boards); Neuroverse, Inc..

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.13/H42

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NSF GRF Award #DGE-0937373

NSF Grant #094158

NIH Grant #5T32GM008339-22

Title: Sensory-motor control underpinnings of a baseline-pointing task in patients with schizophrenia and in neurotypical controls

Authors: *J. NGUYEN, S. M. SILVERSTEIN, T. V. PAPATHOMAS, E. B. TORRES; Grad. Program in Neurosci., Rutgers Univ., New Brunswick, NJ

Abstract: Little is known about sensory-motor integration in patients with schizophrenia (SZ). SZ is a disorder characterized by hallucinations, delusions, perceptual abnormalities, and cognitive dysfunctions. Although motor disturbances have been reported in the literature, how these symptoms relate and translate to sensory motor processes is largely unexplored (Kent et al., 2012). Moreover, clinical assessments of SZ often rely on subjective observational inventories, such as PANSS and DSM-V, to diagnose a widely heterogeneous population. It is therefore imperative to develop objective metrics that can help subtype levels of severity within SZ to serve as biomarkers for better diagnosis and treatment strategies. We examine the signatures of motor output variability of continuous reaching motions in patients with SZ in relation to typically developing controls. An established baseline-pointing task is implemented to look for underlying micro-movement motor signatures using a novel statistical platform for behavioral analyses (Torres, 2011, 2013; Torres et al., 2014). Specifically, we study motions with different levels of intent: the goal-directed reach deliberately aimed at the target, and the transitional, uninstructed retraction of the arm as subjects bring their hand to rest. The signal-to-noise patterns embedded in the motor output variability of these continuous motions are a form of kinesthetic re-afference. From trial to trial, their modulation and central control depend on the continuous returning afferent stream, which those motions themselves cause. Current research indicates that these kinesthetic parameters detect disruptions in proprioception that were previously overlooked in neurological disorders such as autism and Parkinson's disease (Torres et al., 2013; Yanovich, et al., 2013). Additionally, the Frontal Systems Behavioral Scale is administered to assess patients' abilities to manage cognitive control processes such as planning and cognitive flexibility. Preliminary results suggest that stochastic signatures of motor output variability in patients with SZ differ from neurotypical controls, particularly in the uninstructed motions that supplement each goal-directed action. These findings help characterize sensory-motor perturbations in SZ that may play a role in the onset and presence of core symptoms of SZ. By identifying these aberrations in sensor-motor processing, we gain a better understanding of the role of sensory-motor integration on executive functions, which can improve existing diagnostic tools and therapeutic interventions.

Disclosures: J. Nguyen: None. **S.M. Silverstein:** None. **T.V. Papathomas:** None. **E.B. Torres:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.14/H43

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: the National Basic Research Program of China (No 2011CB707800)

Title: The augmentation effect of electroconvulsive therapy on the antipsychotic action of medications and functional corticostriatal connectivity in patients with schizophrenia

Authors: *P. LI[1], R.-J. ZHAO[2], L. SHI[1,3], S.-Q. MENG[1,3], H.-Q. SUN[1], L. LU[1,3];
[1]Peking Univ. Sixth Hosp., Beijing, China; [2]Dept. of Alcohol and Drug Dependence, Beijing Hui-Long-Guan Hospital, Peking Univ., Beijing, China; [3]Natl. Inst. on Drug Dependence, Peking Univ., Beijing, China

Abstract: Background: Studies have demonstrated that combination of electroconvulsive therapy (ECT) and antipsychotic drugs may be more effective than drugs alone for patients with schizophrenia. However, its precise mechanism of action is stillunclear. Functional dysconnectivity of corticostriatal circuitry has been implicated as a risk phenotype for psychosis. Furthermore, the antipsychotic effects of drug treatment might result from the increased functional connectivity of the striatum with prefrontal and limbic regions. In the present study, we used resting-state functional magnetic resonance imaging (rs-fMRI) to explore the longitudinal effect of combined use of ECT and antipsychotics on functional corticostriatal circuitry in patients with schizophrenia. Methods: Twenty nine patients with schizophrenia and 30 healthy control subjects were included in this study. Patients with schizophrenia were then randomly assigned into two groups, the antipsychotics group (n=16) and ECT plus antipsychotics group (n=13). Functional connectivity of striatal regions was examined via functional magnetic resonance imaging using a seed-based approach, and patients were scanned at baseline and after 6 weeks of treatment. Results: The study finally included 39 participants (ECT plus antipsychotics group, n = 10; antipsychotics group, n =15; healthy control group, n=30). Both treatments lowered the Positive and Negative Syndrome Scale (PANSS) scores of the patients, and the combination of ECT and antipsychotics seems to be more effective against positive symptoms than antipsychotics alone ($p=0.08$). ANOVAs and subsequent post hoc t tests demonstrated significant between-group differences in functional connectivity between ventral striatum and anterior cingulate cortex, prefrontal cortex, and Inferior parietal lobule ($p<0.001$, $k>5$). The analysis also revealed a main effect of ECT plus antipsychotics on brain connectivity. The amelioration of positive symptoms by ECT plus antipsychotics was significantly correlated with increased neural activity in orbital prefrontal cortex. There was no discernible difference between groups on global cognition. Conclusion: We demonstrated that drug treatment and ECT produced antipsychotic effects by modulating corticostriatal activity, and ECT augmented the effects of antipsychotics via increasing the functional connectivity between prefrontal cortex and ventral striatum. These findings contribute to understanding the mechanisms underlying the enhancing effect of ECT on the antipsychotic actions of medications.

Disclosures: **P. Li:** None. **R. Zhao:** None. **L. Shi:** None. **S. Meng:** None. **H. Sun:** None. **L. Lu:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.15/H44

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Fondecyt 11140464

Conicyt-PCHA Doctorado Nacional 2014-21141021

Biomedical Neuroscience Institute

Title: Processing bimodal altered in individuals at high risk for psychosis: An ERP study

Authors: ***M. B. ABURTO**[1,2], R. CASTILLO[2], S. CORRAL[2], R. MAYOL[2], V. DE ANGEL[2], D. GONZÁLEZ[2], M. J. VILLAR[2], J. CORTÉS-BRIONES[3], H. SILVA[2], P. GASPAR[2,4]; [1]Univ. of Chile, Santiago, Chile; [2]Translational Psychiatry Laboratory, Dept. of Psychiatry and Mental Health, Clin. Hospital, Univ. of Chile, Santiago, Chile; [3]Yale University, West Haven, CT, United States, Santiago, Chile; [4]Biomed. Neurosci. Inst., Santiago, Chile

Abstract: Alterations in unimodal perception are symptoms frequently observed in patients with schizophrenia and individuals with the ultra high risk (UHR) syndrome of psychosis. However, the alteration in multimodal stimulus integration has not been as evaluated in patients with UHR. This multimodal integration can be studied through sensory stimuli that generate a sensory illusion, such as the double flash illusion. This occurs when a flash of light is presented between two auditory stimuli separated by 60- 100 ms - in this situation healthy people report seeing a second flash. The characteristic components of event-related potentials (ERP) evoked by this stimulus are a positive component between 60-120 ms (PD180) and a negative component between 252-284 ms (ND270). In this study we performed an ERP analysis by means of a 64-channel electroencephalogram during the execution of the double flash task in UHR subjects and healthy controls (HC). Subjects with UHR syndrome (N = 6) showed both behavioral and ERP alterations in the characteristic components of the double flash illusion. The UHR group showed a lower percentage of correct answers in the perception of the double flash illusion compared to HC. An increase in the latency of emergence of the ERP evoked by this stimulus was also observed, that is, components PD180 and ND270. These results suggest an altered processing of bimodal stimuli in the early stages of the disease. The study of multimodal alterations can be a potential biomarker for early detection in psychosis.

Disclosures: **M.B. Aburto:** None. **R. Castillo:** None. **S. Corral:** None. **R. Mayol:** None. **V. de Angel:** None. **D. González:** None. **M.J. Villar:** None. **J. Cortés-Briones:** None. **H. Silva:** None. **P. Gaspar:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.16/H45

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Prevalence of psychoactive substance use and its effects on mental health of students of abubakar tafawa balewa university, bauchi, Nigeria

Authors: *Y. M. MAHMUD[1], K. A. AYANDA[2], D. SULYMAN[2];
[2]Psychiatry, [1]Abubakar Tafawa Balewa Univ. Teaching Hospita, Bauchi, Nigeria

Abstract: Alcohol and substance use are prevalent in this part of the world. Excessive alcohol intake and use of illicit drugs and substances of addiction have been associated with increased psychiatric morbidity and psychosocial problems in different populations. Studies had shown relationship between substance uses and different types of psychiatric disorders such as mood disorders, anxiety disorders, psychotic disorders as well as dementias. These disorders have been divided into substance use disorders and substance related disorders. Psychosocio-economic problems resulting from use of substances could be family discord, unemployment, conflict with law and financial difficulties. Studies have been done on the use and effects of psychoactive substances on mental health of adolescents and young adults in different settings such as motor park, schools and community. There is dearth of studies in this part of the country especially with the increase level of insecurity in this part of the country and involvement of youth in this act of violence. This present study therefore aimed to find the prevalence of psychoactive substance use among the students of Abubakar Tafawa Balewa University, Bauchi, Nigeria. It also examined types of psychiatric disorders among them and factors associated with the presence of psychiatric morbidities among youths using psychoactive substances. Pro formal questionnaire designed to obtain the socio-demographic and risk factors for psychiatric morbidity from the respondents and student substance use surveys questionnaire was administered to the students to assess their psychoactive substance use among the respondents. They were also required to complete the twelve-items, General Health Questionnaire (GHQ-12). Of the one thousand, forty seven students of the institutions given questionnaire, nine hundred and eighty three respondents returned completed questionnaires. Part of the result shows there is high prevalence of substance use among the students which likely predispose to their mental health problems. Research is still ongoing to evaluate the determinant factors for the use of these drugs and the predominant psychiatric disorder due to a particular psychoactive substance use.

Disclosures: **Y.M. Mahmud:** None. **K.A. Ayanda:** None. **D. Sulyman:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.17/H46

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Fondecyt

Conicyt

Title: Aberrant activity in coherent motion perception at high risk Psychosis subjects

Authors: ***R. MAYOL**[1,2], B. ABURTO[1,2], R. CASTILLO[1,2], S. CORRAL[1,2], D. GONZÁLEZ[1,2], V. DE ANGEL[1,2], J. CORTES-BRIONES[3], H. SILVA[1,4], P. GASPAR[1,2]; [1]Univ. De Chile, Santiago, Chile; [2]Clínica Psiquiátrica Universitaria, Santiago, Chile; [3]Yale University, Dept. of Psychiatry, New Haven, CT; [4]Clínica Psiquiátrica Universitara, Santiago, Chile

Abstract: Visual Motion processing involves an integration of a distributed neural activity from long range brain areas. People with schizophrenia (Sz) have an impairment in the discrimination and detection of different kinds of motion processing such as coherent motion and biological motion. Current theories of schizophrenia have posited that abnormalities in coherent motion integration rely in difficulties in sensory processing. Little is known about the neural substrates of coherent motion perception in first episode and high risk psychosis. We hypothesized that subjects at high risk for psychosis (HRP) show deficits in the perception of coherent motion associated with late aberrant oscillatory brain activity. This research studied the electroencephalogram (EEG) activity in six high risk individuals and their corresponding healthy controls. The experiment included three motion conditions: coherent motion 100%, coherent motion 50%, incoherent motion and stationary. In the coherent condition, all dots moved from left to right within the stimuli window, and during the incoherent condition, all dots moved at randomly generated angles. Three ERPs were examined: P1, N1, and a late positive potential (LPP). In the control group we found no significant differences in ERP's P1 and N1 for within group comparison of all motion conditions studied. Moreover, a significant increase was found in the LPP ERP during the coherent motion conditions. However, the high risk group showed an aberrant pattern in the amplitude of LPP which is only elicited in the 50% coherence motion condition. These findings suggest that the behavioral dysfunctions in motion perception could be associated with late aberrant oscillatory brain activity a failure observed in LPP. These results allow an improvement in markers for early diagnosis in adults with psychosis.

Disclosures: **R. Mayol:** None. **B. Aburto:** None. **R. Castillo:** None. **S. Corral:** None. **D. González:** None. **V. De Angel:** None. **J. Cortes-Briones:** None. **H. Silva:** None. **P. Gaspar:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.18/H47

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Early integration of visual and audio-visual information in schizophrenia as assessed by contour integration and sound induced flash illusion paradigms

Authors: *H. TURKOZER[1], E. KALE[2], Z. PAMIR[3], H. BOYACI[4], D. ONGUR[5], V. TOPCUOGLU[1];
[1]Dept. of Psychiatry, Marmara Univ., Istanbul, Turkey; [2]Brain Res. Ctr., Ankara Univ., Ankara, Turkey; [3]Neurosci. Grad. Program, [4]Dept. of Psychology, Bilkent Univ., Ankara, Turkey; [5]Dept. of Psychiatry, Harvard Univ. and McLean Hosp., Belmont, MA

Abstract: Visual perceptual organization and multi-sensory integration deficits have been observed in schizophrenia and are suggested to be related to the NMDA receptor hypofunction and reduced connectivity. Evidence suggests that both visual contour integration and sound induced flash illusion -an audio-visual integration paradigm- are associated with the activity of early visual areas (V1-V4). Here, we investigated integration deficits in schizophrenia in early levels of sensory processing, using a variant of contour integration paradigm, Jittered Orientation Visual Integration (JOVI) task and sound induced flash illusion. 18 patients with schizophrenia and 22 healthy control subjects participated in the study. Patients with schizophrenia showed significantly lower performance in the JOVI task than control subjects, which is consistent with prior studies. Contour integration performance was found to be negatively correlated with overall symptom severity and active social avoidance in patients, as measured by the Positive and Negative Syndrome Scale. We also demonstrated that poorer contour integration performance was associated with negative, disorganized and overall schizotypal traits in control subjects, as assessed by the Schizotypal Personality Questionnaire. No significant difference in illusory response rates for sound induced flash fission or fusion illusions were found between patients with schizophrenia and healthy controls. Sound induced flash illusion perception was not associated with symptom severity in patients or with schizotypal traits in the control group. No correlations were found between contour integration performance and sound induced flash

illusory response rates. These results suggest that multi-sensory convergence at early stages of visual processing is intact in schizophrenia although visual perceptual organization, another integration function of early visual cortex, is shown to be deficient. These data also suggest that JOVI task performance, which is associated both with symptom severity and schizotypal traits, has both state and trait-dependent characteristics.

Disclosures: H. Turkozer: None. **E. Kale:** None. **Z. Pamir:** None. **H. Boyaci:** None. **D. Ongur:** None. **V. Topcuoglu:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.19/H48

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Brain/MINDS, AMED, Japan

Title: Mismatch negativity is associated with plasma levels of glutamate in patients with first-episode psychosis

Authors: *D. KOSHIYAMA[1], T. NAGAI[1], K. KIRIHARA[1], M. TADA[1], S. KOIKE[2], M. SUGA[1], T. ARAKI[3], K. KASAI[1];
[1]Dept. of Neuropsychiatry, Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan; [2]Office for Mental Hlth. Support, the Univ. of Tokyo, Tokyo, Japan; [3]Dept. of Youth Mental Health, Grad. Sch. of Medicine, Univ. of Tokyo, Tokyo, Japan

Abstract: Background: Mismatch negativity (MMN) is one of the event-related potentials. Many studies have shown that MMN amplitude is reduced in psychotic disorders and associated with cognitive dysfunction. Reduced MMN amplitude may reflect dysfunction of NMDA receptors in psychotic disorders because NMDA receptor antagonists reduce MMN amplitude. Therefore, MMN might be a marker that links molecular pathology to dysfunction in clinical settings. However, there are few studies to investigate a relationship between MMN and molecular pathology in patients with psychotic disorders. In this study, we examined plasma levels of glutamate, MMN amplitude, and cognitive functions to investigate their relationships in patients with psychotic disorders. We focused on early stages because clinical stages of psychotic disorders affect MMN amplitude and early stages are important periods for functional outcome of patients with psychotic disorders. Methods: Participants consisted of 19 patients with first-episode psychosis (FEP), 21 ultra-high risk (UHR) individuals, and 16 healthy control (HC) subjects. We measured plasma levels of glutamate and MMN amplitude. We also measured cognitive functions by BACS-J (Brief Assessment for Cognition in Schizophrenia Japanese version). Results: In FEP compared to HC, plasma levels of glutamate increased, MMN

amplitude was reduced, and cognitive functions were impaired. In FEP, MMN amplitude was positively correlated with plasma levels of glutamate and negatively correlated with cognitive functions. In UHR compared to HC, MMN amplitude was reduced. In UHR, MMN amplitude was significantly correlated with neither plasma levels of glutamate nor cognitive functions. Conclusion: FEP showed increased plasma levels of glutamate, reduced MMN amplitude, and impaired cognitive functions compared HC. Reduced MMN amplitude was associated with high plasma levels of glutamate and cognitive dysfunction in FEP. These findings suggest MMN can be a marker that links molecular pathology to dysfunction in clinical settings in early stages of psychotic disorders.

Disclosures: **D. Koshiyama:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan. **T. Nagai:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan. **K. Kirihara:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan. **M. Tada:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan. **S. Koike:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan. **M. Suga:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan. **T. Araki:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan. **K. Kasai:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain/MINDS, AMED, Japan.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.20/I1

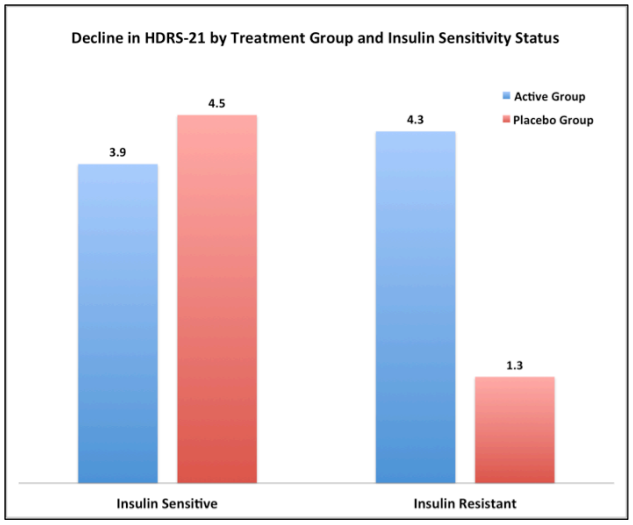
Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

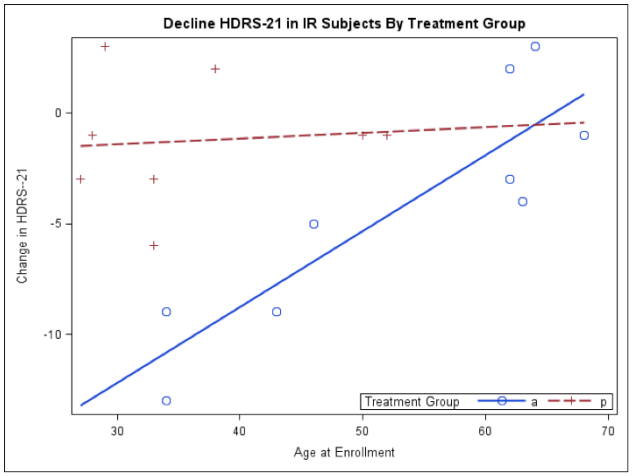
Support: R21 MH093948-01A1

Title: Adjuvant pioglitazone for unremitted depression: clinical correlates of treatment response

Authors: K. WATSON LIN[1], T. WROOLIE[1], *N. L. RASGON[2];
[1]Stanford Univ., Stanford, CA; [2]Stanford Sch. of Medicine, Stanford Univ., Palo Alto, CA

Abstract: Abstract Background: Previous studies suggest that insulin-sensitizing agents could play a significant role in the treatment of major depression, particularly depression in patients with documented insulin resistance or those who are resistant to standard psychopharmacological approaches. This study aimed to assess the effects on depressive symptoms with adjuvant treatment with the PPARγ-agonist pioglitazone. Methods: Patients (N=37) with non-remitting depression receiving standard psychiatric regimens for depression were randomized across an insulin sensitivity spectrum in a 12-week double blind, randomized controlled trial of pioglitazone or placebo. Results: Improvement in depression was associated with improvement in glucose metabolism but only in patients with insulin resistance. An age effect was also shown in that response to pioglitazone was more beneficial in younger aged patients. Conclusion: Study findings suggest differential improvement in depression severity according to both glucose metabolic status and level of depression at baseline. A greater understanding of the reciprocal links between depression and IR may lead to a dramatic shift in the way in which depression is conceptualized and treated, with a greater focus on treating and/or preventing metabolic dysfunction.





Disclosures: **K. Watson Lin:** A. Employment/Salary (full or part-time):; Stanford University. **T. Wroolie:** A. Employment/Salary (full or part-time):; Stanford University. **N.L. Rasgon:** A. Employment/Salary (full or part-time):; Stanford University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Magceutics Inc., Corcept. F. Consulting Fees (e.g., advisory boards); Shire Pharmaceuticals, Shire Pharmaceuticals.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.21/I2

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Off-label use of transmucosal ketamine as a rapid acting antidepressant: a retrospective chart review

Authors: *L. NGUYEN[1,2], C. B. WEAVER[1], K. J. CRAMER[3], S. E. POLLARD[2], P. J. MARSHALEK[2], R. R. MATSUMOTO[1,2,4];
[1]Pharmaceut. Sci., WVU Sch. of Pharm., Morgantown, WV; [2]Behavioral Med. and Pyschiatry, WVU Sch. of Med., Morgantown, WV; [3]WVU Schoof of Nursing, Morgantown, WV; [4]Touro Univ. CA Col. of Pharm., Vallego, CA

Abstract: The N-methyl-D-aspartate (NMDA) receptor antagonist ketamine administered at sub-anesthetic doses via an intravenous (IV) route can elicit robust, rapid and long-lasting antidepressant effect, even in treatment resistant patients. However, the need for IV administration limits the use of ketamine to an inpatient setting, requiring support services and monitoring. To minimize the adverse events associated with IV ketamine, including acute

dissociative and psychotomimetic effects and cardiovascular changes, recent investigations have attempted alternative routes of administration to elicit rapid antidepressant effects. This study evaluated the effectiveness and safety of sub-anesthetic doses of ketamine using an off-label, transmucosal administration route in patients with treatment resistant depression. A retrospective chart review was conducted to identify patients who met the inclusion criteria for treatment resistant major depressive disorder (MDD). Seventeen such patients who received sub-anesthetic doses of ketamine were included. Patient demographics, efficacy (drug refill, clinician notes), side effects, and concurrent medications were assessed. Benefit from low-dose transmucosal ketamine was noted in 76% of subjects (average age 48, 88% female), with a dose duration lasting 10-14 days. No notable side effects were noted. The most common classes of concurrent medications to which ketamine was added were serotonin norepinephrine reuptake inhibitors (59%), stimulants (47%), folate replacement (47%), and benzodiazepines (47%). Our results provide preliminary evidence of the effectiveness and safety of low-dose transmucosal ketamine in treatment resistant MDD patients. Formal prospective clinical trials are warranted to validate these findings.

Disclosures: **L. Nguyen:** None. **C.B. Weaver:** None. **K.J. Cramer:** None. **S.E. Pollard:** None. **P.J. Marshalek:** None. **R.R. Matsumoto:** None.

Poster

311. Major Mental Disorders: Clinical Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 311.22/I3

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Cooling the forebrain in adults

Authors: *B. BARRERA-MERA;
Fac Med, UNAM, Mexico 04510 DF, Mexico

Abstract: The cooling of forebrain throughout the application of iced water on the skin of frontal and parietal regions, has been used to suppress of the consequences of mental fatigue, the major discomfort induced by the excessive ingestion of alcohol and also to relief of a paranoid syndrome. In all the cases the time of application of such a remedy lasted since minutes to some hours. This resource which is not only easy to applied but additionally innocuous and inexpensive, could be use to remedy some other mental pathologies i.e. depression, distress, and inclusively the severe changes in personality of that patients that suffers of inhalant addictions. .Presently we are intent to use the remedy in the last patients not by minutes or hours but during several days

Disclosures: **B. Barrera-Mera:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.01/I4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH 1R01 MH093723

Title: Development of a genetic risk score to index the integrity of somatostatin-positive GABA neurons in human cortex

Authors: *Y. S. NIKOLOVA[1,2], J. PIPITONE[2,3], E. SIBILLE[2,4];
[1]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Campbell Family Mental Hlth. Res. Inst. of CAMH, Toronto, ON, Canada; [3]Kimel Family Translational Imaging-Genetics Res. Lab, Res. Imaging Ctr., CAMH, Toronto, ON, Canada; [4]Departments of Psychiatry, Pharmacol. and Toxicology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Neurons expressing somatostatin (SST) constitute 20-30% of all GABA neurons in the human brain and mediate dendritic inhibition throughout the cortex. Results from studies in animal models and post-mortem human brain tissue suggest these neurons are impaired in depression and may be selectively vulnerable to stress. This vulnerability is reflected in changes in the neurons' transcriptomes potentially reflecting an increased susceptibility to endoplasmic reticulum and oxidative stress. Little is known about inter-individual variability in the functioning of these neurons in living humans. Common functional genetic variation can be used to bridge basic molecular and human *in vivo* work. Therefore, we sought to create a genetic risk score (GRS), using a gene co-expression network approach to index the relative integrity of SST-positive neurons based on combined post-mortem gene expression (mRNA) and single nucleotide polymorphism (SNP) data. Post-mortem gene expression and SNP data were available for 180 Caucasian individuals free of neuropsychiatric illness (35 women, mean age: 51.68 ± 14.64; range 16-96) and two brain regions - Brodmann areas 11 and 47 (BA11 and BA47). To create a gene co-expression network for SST, we selected the top 200 genes positively correlated with SST across both regions, after covarying for post-mortem interval, brain pH, RNA integrity number, and gender. To ensure the specificity of our results to SST neurons, we conducted parallel co-expression analyses for other subpopulations of GABA neurons expressing parvalbumin, cholecystokinin, and vasoactive intestinal peptide, and removed overlapping genes. To validate the resulting SST-specific co-expression network, we conducted a Gene Ontology (GO) term enrichment analysis using the Gene Ontology enRIchment anaLysis and visuaLizAtion tool (GOrilla; http://cbl-gorilla.cs.technion.ac.il/). Out of the top 200 genes showing a positive association with SST across both BA11 and BA47, 83 were uniquely co-expressed with SST. Among those, a GO term analysis demonstrated an enrichment of genes related to GABA A receptor function (p=7.84 x 10-5; genes: GABRB3, GABRA5, GABRA3),

consistent with the involvement of SST neurons in dendritic inhibition, and NADH dehydrogenase activity (p=6.42 x 10-4; genes: NDUFB7, NDUFS6, NDUFV1), consistent with a link between SST function and oxidative cellular processes. These preliminary results provide partial validation for our gene co-expression network approach. In a next step, SNPs regulating the expression of genes co-expressed with SST will be used to construct a GRS, which will subsequently be applied to *in vivo* neuroimaging data.

Disclosures: Y.S. Nikolova: None. **J. Pipitone:** None. **E. Sibille:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.02/I5

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: 2 R01 MH083862 06A1

1 P50 MH090964-01A1

AFSP Standard Research Grant

BRAIN & BEHAVIOR RESEARCH FOUNDATION Independent Investigator Grant

Title: In subjects with major depressive disorder CREB expression in the dentate gyrus correlates with number of granule neurons and decreases with aging

Authors: L. BONILLA[1], R. HEN[2], A. J. DWORK[2], G. B. ROSOKLIJA[2], V. ARANGO[2], J. J. MANN[2], *M. BOLDRINI[2];
[1]Barnard Col., New York, NY; [2]Psychiatry - Mol. Imaging and Neuropathology, Columbia Univ. - NYSPI, New York, NY

Abstract: Increased adult neurogenesis improves pattern separation (Sahay et al., 2011). In MDD there is a deficit in attention disengagement from negative material (Foland-Ross and Gotlib, 2012), with biased memories and judgments (Lemoult et al., 2012). Hippocampal atrophy is found even in first-episode MDD (Cole et al., 2011). Selective serotonin reuptake inhibitors (SSRI) increase neurogenesis in human DG (Boldrini et al, 2009, 2012) with unknown signaling mechanisms. The cAMP responsible element-binding (CREB) protein is a leucine zipper transcription factor (Borrelli et al., 1992) downstream of neurotrophin receptors, G-protein-coupled receptors and others (Carlezon et al, 2005), has a role in learning and memory (Silva et al, 1998), antidepressant action (Nibuya et al, 1996) and antidepressant-like effects (Chen et al, 2001). Conversely, mice with hippocampal CREB deletion respond faster to antidepressants and have increased neurogenesis (Gur et al, 2007), possibly due to up-regulation of CREB-family protein cAMP response-element modulator (CREM) (Gunderson et al, 2013). To assess

mechanisms of SSRI action on human adult neurogenesis, we quantified using immunohistochemistry and stereology CREB+ GN and glial cells in DG brain tissue from SSRI-treated (N=12), and untreated subjects with MDD (N=24) and controls with no psychiatric disease or treatment (N=24). We also assessed numbers of NPCs (nestin+), neuroblast (PSA-NCAM+) and GNs (NeuN+), and correlated their umber with CREB+ GNs and glial cells in the DG. Clinical data were obtained using psychological autopsy (Kelly and Mann, 1996), toxicology and neuropathology exams performed on all samples. Number of CREB+ cells decreased with increasing age in untreated MDD (r2= .778, F=17.503, p=.009), but not in controls and MDD*SSRI. In anterior DG, number of CREB+ GNs correlate with total number of GNs in untreated MDD (r2= .757, F=15.553, p=.011) and MDD*SSRI (r2= .703, F=11.824, p=.018), but not in controls. In anterior DG, CREB+ GNs were more in untreated MDD vs. controls (p=0.027) and MDD*SSRI ($p<0.05$). No group differences were found for CREB+ glial cell number. Fewer CREB+ GNs in older MDD suggests slower transcription processes. The correlation between CREB expression and GN total number in MDD suggests more maturation and or survival with more CREB expression. More CREB+ GNs in untreated vs. treated MDD and controls may be due to the fact that we are measuring non-phosphorylated CREB or to a compensating response or to an interaction with CREM that needs to be dissected. Further studies on CREB expression in NPCs and neuroblasts are needed to understand the regulation of neurogenesis by CREB.

Disclosures: **L. Bonilla:** None. **R. Hen:** None. **A.J. Dwork:** None. **G.B. Rosoklija:** None. **V. Arango:** None. **J.J. Mann:** None. **M. Boldrini:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.03/I6

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Vahlteich Scholar Award (MS)

American Foundation for Suicide Prevention

Title: Sex differences in glutamate receptors in major depression and suicide

Authors: ***M. S. SODHI**[1], A. GRAY[2], A. DEEP-SOBOSLAY[3], T. HYDE[3], J. KLEINMAN[3]; [1]Col. of Pharm., Univ. of Illinois At Chicago, Chicago, IL; [2]Pharm. Practice, UIC, Chicago, IL; [3]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Accumulating data indicate that the glutamate system is disrupted in major depression (MDD) and recent clinical research suggests that ketamine, an antagonist of the NMDA glutamate receptor, has rapid antidepressant efficacy. Here we report findings from gene

expression studies of a large cohort of postmortem subjects, including subjects with MDD and controls. Our data reveal higher expression levels of the majority of glutamatergic genes tested in the dorsolateral prefrontal cortex (DLPFC) in MDD ($F_{21, 59}=2.32$, $p=0.006$). Post-hoc data indicate that these gene expression differences occurred mostly in the female subjects. Higher expression levels of GRIN1, GRIN2A-D, GRIA2-4, GRIK1-2, GRM1, GRM4, GRM5 and GRM7 were detected in the female patients with MDD. In contrast, GRM5 expression was lower in male MDD patients relative to male controls. When MDD suicides were compared with MDD non-suicides, GRIN2B, GRIK3 and GRM2 were expressed at higher levels in the suicides. In addition, the suicides also had higher levels of GRIA2 RNA editing. Higher expression levels were detected for several additional genes but these were not statistically significant after correction for multiple comparisons. In summary, our analyses indicate a generalized disruption of the regulation of the GluRs in the DLPFC of females with MDD, with more specific GluR alterations in the suicides and in the male groups. These data reveal further evidence that in addition to the NMDA receptor, the AMPA, kainate and the metabotropic GluRs may be targets for the development of rapidly acting antidepressant drugs.

Disclosures: **M.S. Sodhi:** None. **A. Gray:** None. **A. Deep-Soboslay:** None. **T. Hyde:** None. **J. Kleinman:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.04/I7

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: R21 MH094888

R01 MH083862 06A1

P50 MH090964-01A1

AFSP Standard Research Grant

BRAIN & BEHAVIOR RESEARCH FOUNDATION Independent Investigator Grant

Title: VEGFR2 is increased in the dentate gyrus of antidepressant treated subjects and correlates with measures of angiogenesis, neurogenesis, and serotonin 1A receptor mRNA

Authors: *A. N. SANTIAGO[1,4], K. B. STEELE[5], T. H. BUTT[6], S. KASSIR[6], M. BAKALIAN[6], V. ARANGO[6,2], A. DWORK[6,2,3], G. ROSOKLIJA[6,2,7], J. MANN[6,2], M. BOLDRINI[6,2]; [2]Dept. of Psychiatry, [3]Departments of Pathology and Cell Biol., [1]Columbia Univ., New York, NY; [4]Ctr. for Neural Studies, New York Univ., New York, NY; [5]Albert Einstein Col. of Med., New York, NY; [6]Mol. Imaging and Neuropathology Div., New York State Psychiatric Inst., New

York, NY; [7]Macedonian Acad. of Sci. & Arts, Skopje, Macedonia, The Former Yugoslav Republic of

Abstract: Adult neurogenesis is hypothesized to be impaired in major depressive disorder (MDD). Angiogenesis and neurogenesis in the hippocampal dentate gyrus (DG) are co-regulated by growth factors, including vascular endothelial growth factor (VEGF) in the neurogeneic niche. VEGF and its primary neural receptor (VEGFR2) have been shown to mediate increased cell proliferation in rodents following electroconvulsive seizure and treatment with selective serotonin reuptake inhibitors (SSRI). It is not known whether SSRIs increase VEGFR2 in the human brain, nor if this is a mechanism by which SSRIs increase neurogenesis. It is also unknown whether the serotonin 1A receptor (HTR1A), which has been implicated in antidepressant-mediated neurogenesis, is up-regulated with VEGFR2 in human subjects. To test the role of VEGFR2 in mediating adult neurogenesis in major depressive disorder (MDD) and in response to SSRI, we used immunohistochemistry and stereology to quantify numbers of VEGFR2-immunoreactive (IR) DG cells and endothelial cells in human postmortem hippocampus from psychiatrically healthy control, untreated MDD (MDD*U), and SSRI-treated MDD subjects (MDD*SSRI). We performed *in situ* quantification of the HTR1A mRNA. Additionally, we correlated VEGFR2-IR cell count with neural progenitor cell (NPC) number, mature granule neuron number, capillary surface area, dentate gyrus volume, and HTR1A mRNA density. We found that MDD*SSRI had a greater number of VEGFR2-IR DG cells than both control ($p = .02$) and MDD*U ($p = .001$) in the anterior DG, but not the mid or posterior DG. VEGFR2-IR endothelial cells in DG capillaries were more in MDD*SSRI vs. control ($p = .043$) and MDD*U (0.014). VEGFR2-IR DG cell number correlated with NPC number ($r2=.299$, $p=.015$), granule neuron number ($r2=.228$, $p = .021$), capillary surface area ($r2=.333$, $p=.010$), anterior DG volume ($r2=.349$, $p=.003$), and anterior DG HTR1A mRNA ($r2=.233$, $p=.020$). Our finding that VEGFR2 cell number is greater in SSRI-treated subjects supports a role for VEGF in antidepressant action. Correlations with NPC number, granule neuron number, and capillary surface area support the hypothesis that VEGFR2 plays an integral role in both angiogenesis and neurogenesis. Increased expression of HTR1A mRNA with VEGFR2-IR cell count suggests that SSRI treatment may induce neurogenesis and angiogenesis by up-regulating HTR1A mediated VEGF signaling. This conclusion is supported by rodent studies, which show that behavioral effects of SSRIs are diminished by VEGFR2 inhibitors.

Disclosures: **A.N. Santiago:** None. **K.B. Steele:** None. **T.H. Butt:** None. **S. Kassir:** None. **M. Bakalian:** None. **V. Arango:** None. **A. Dwork:** None. **G. Rosoklija:** None. **J. Mann:** None. **M. Boldrini:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.05/I8

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Fonds de recherche du Québec - Santé

Canadian Institutes of Health Research

Title: Oxytocin: A neurohormone link to the epigenetics of early life adversity and suicide

Authors: *D. ALMEIDA[1,2], L. FIORI[1], G. TURECKI[1,2,3,4];
[1]McGill Group For Suicide Studies, Verdun, QC, Canada; [2]Neurol. & Neurosurg., [3]Psychiatry, [4]Human Genet., McGill Univ., Montreal, QC, Canada

Abstract: Oxytocin is a mammalian neurohypophysial hormone which acts primarily as a neuromodulator in the CNS. The early development of secure attachments, relationship quality, and the ability to regulate and manage emotions are all instances of psychological resources influenced by the oxytocinergic system. Previous studies have shown that early life adversity might act to disturb the oxytocinergic system during critical developmental periods. A body of literature also supports alterations in the oxytocinergic system as a predisposing factor for suicidal behaviour. Our research looks into the expression of genes regulating the oxytocinergic system in the prefrontal cortex of suicide completers with a history of abuse, non-abused suicide completers, and healthy controls. Expression data from the prefrontal cortex of these subjects indicates an effect of abuse on genes involved in oxytocin metabolism and function. Specifically, suicide completers with a history of abuse show a significant upregulation of LNPEP (an enzyme responsible for the breakdown of neuropeptides in the brain), OXTR (oxytocin receptor), and AVPR1B (arginine vasopressin receptor 1 B), when compared to non-abused suicide completers and healthy controls. Recently, several studies have identified epigenetic mechanisms influencing the oxytocinergic system, with an emphasis on methylation. We are therefore also investigating methylation via targeted bisulfite sequencing of CpG rich regions within LNPEP, OXTR, & AVPR1B. The practical goals of this research are two fold, first they seek to strengthen the literature on child-abuse and CNS changes in psychiatric disorders, and secondly, to determine whether epigenetic modification of the oxytocinergic system represents a CNS biomarker of early life adversity and later suicidal behaviour.

Disclosures: D. Almeida: None. **L. Fiori:** None. **G. Turecki:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.06/I9

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: CONACyT grants No. 138663

CONACyT grants No. 129303

Title: Increase in the nitration levels and astrocytes in the Prefrontal Cortex of suicide victims

Authors: *F. GARCIA-DOLORES[1], F. E. TAKAHASHI[2], R. C. MENDOZA MORALES[3], R. A. VAZQUEZ-ROQUE[4], A. D. DÍAZ FONSECA[5], F. DE LA CRUZ LÓPEZ[6], G. FLORES[7]; [1]Patologia, Inst. De Ciencias Forenses, Ciudad DE Mexico, Mexico; [2]Pathology, Inst. de Ciencias Forenses, Mexico, D.F., Mexico; [3]Pathology, Inst. de Ciencias Forenses, Mexico, D. F., Mexico; [4]Inst. de Fisiología, Benemerita Uniersidad Autonoma de Puebla, Puebla, Puebla, Mexico; [5]Facultad de Ciencias Químicas, Benemerita Univ. Autónoma de Puebla, Puebla, Publa, Mexico; [6]Escuela Nacional de Ciencias Biologicas, Inst. Politecnico Nacional, Mexico, D.F., Mexico; [7]Inst. de Fisiologia, Benemerita Universida Autonoma de Puebla, Puebla, Puebla, Mexico

Abstract: The World Health Organization (WHO) has been estimated that every 40 seconds one person commits suicide in one part of the world, in 2012 WHO estimated that nearly 1 million people die from suicide yearly. For these reasons, suicide has become a public health problem. Suicide risk is influenced by a wide range of factors such as the presence of psychopathologies like major depressive disorder, alcoholism, drug abuse, childhood adversity and stressful life events, all this exposure to stress can produce long lasting changes in brain circuits, behaviors and epigenetic behaviors. Some studies have reported an increase in the DNA methylation levels on the orbital prefrontal cortex. It is know that one of the brain region involved in the regulation of stress is the prefrontal cortex. Other results suggest Corticotropin-releasing hormone levels were elevated in frontopolar and dorsomedial prefrontal cortex, but not in the ventrolateral prefrontal cortex of suicide victims. Human tissue specimens were obtained according to an institutional approved protocol Tissue was coded and samples had no person identifiers. The brains were collected at forensic autopsies. With informed consent, family members were interviewed as part of a psychological autopsy to obtain demographic information and any evidence of psychiatric or somatic disease. Depressed-suicide cases were selected according to the following criteria: death by suicide, diagnosis of major depressive disorder *(DSMIV),* absence of psychotropic or illegal drugs on toxicological screens, death without prolonged agonal state or protracted medical illness. Controls did not have an Axis I psychiatric disorder, were drug-free and died suddenly without a prolonged agonal period from causes other than suicide. The aim of the present study was measure the changes in neuronal density by stereological processes, correlating by the measure of GFAP and protein nitration, in tissues of suicide victims in PFC. Our results suggest that suicide victims have a decrease in neuronal density and an increasing in astocytes and protein nitration in comparation to the control group. In conclusion the changes found in brains of suicide victims suggest a disruption in the neurons of the PFC and an inflammation process in the region. (Supported by: CONACyT grants No. 138663 and 129303 to G Flores).

Disclosures: **F. Garcia-Dolores:** None. **F.E. Takahashi:** None. **R.C. Mendoza Morales:** None. **R.A. Vazquez-Roque:** None. **A.D. Díaz Fonseca:** None. **F. De la Cruz López:** None. **G. Flores:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.07/I10

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Lundbeck Research USA

Title: Association between kynurenine metabolism and transcripts related to neuroinflammatory signaling in postmortem human brain

Authors: ***A. POCIVAVSEK**[1], A. W. LEE[2], A. ADBOURAHMAN[2], K. V. SATHYASAIKUMAR[1], J. A. TAMM[2], F. M. NOTARANGELO[1], J. WIEDEMANN[3], T. MÖLLER[2], G. TURECKI[4], R. SCHWARCZ[1], B. CAMPBELL[2];
[1]Maryland Psychiatric Res. Center, Dept of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Lundbeck Res. USA, Paramus, NJ; [3]Lundbeck, Valby, Denmark; [4]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Inflammatory cytokines and subsequent dysregulation of kynurenine pathway (KP) metabolism may contribute to the development of depressive symptoms in a subset of major depressive disorder (MDD) patients. Though increased kynurenine production and elevated proinflammatory mediators in serum and plasma are relatively common findings in MDD studies, fewer reports have evaluated neuroinflammation or KP dysregulation in postmortem brain. A small number of studies reported elevated levels of quinolinic acid in the brain or cerebrospinal fluid in suicide attempters or victims (Erhardt S et al., 2013. Neuropsychopharmacology 38: 743-52; Steiner J et al., 2011. J Neuroinflammation 8: 94-102; Sublette ME et al., 2011. Brain Beh Immun 25: 1272-78). To better understand the role of inflammation and KP metabolism in MDD, the current study examined levels of KP metabolites, activity and expression of KP enzymes, as well as expression of neuroinflammation-relevant genes in dorsolateral prefrontal cortex from individuals with MDD who died of natural causes, those with MDD who died from suicide, and non-MDD controls. Levels of tumor necrosis factor receptor superfamily member 1A (TNFRSF1A) were higher in brains of MDD donors who died of natural causes compared to non-MDD controls. Moreover, levels of tumor necrosis factor alpha were lower in brains from donors who died from suicide compared to non-MDD controls, while there were no differences between groups in any of the other genes measured. Interestingly, we did not find significant differences between groups in KP metabolites and enzymes. However, there were correlations between levels of KP metabolites and several of the

transcripts associated with neuroinflammatory signaling, including interleukin-1 receptor (ILR1), TNFRSF1A, myeloid differentiation primary response gene 88 (MYD88), nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1), and toll-like receptor 2 (TLR2). The expression of the KP enzymes kynureninase and 3-hydroxyanthranilate 3,4-dioxygenase was also positively correlated with transcripts associated with neuroinflammatory signaling, including interferon regulatory factor 9 (IRF9), macrophage migration inhibitory factor (MIF), toll like receptor 4 (TLR4), translocator protein (TSPO), ionized calcium-binding adapter molecule (IBA1) and am subunit of Mac-1 (CD11B). Thus, while our study did not reveal differences in KP metabolism, the data show that regardless of group status (e.g. control vs. MDD), markers indicative of microglial priming are associated with brain KP metabolites and enzymes within the quinolinic acid branch of the KP.

Disclosures: **A. Pocivavsek:** None. **A.W. Lee:** None. **A. Adbourahman:** None. **K.V. Sathyasaikumar:** None. **J.A. Tamm:** None. **F.M. Notarangelo:** None. **J. Wiedemann:** None. **T. Möller:** None. **G. Turecki:** None. **R. Schwarcz:** None. **B. Campbell:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.08/I11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R21 MH083175 J

Pritzker Neuropsychiatric Research Disorders Consortium

NIH T-32-NS076401

Title: The microRNA regulatory network is altered in anterior cingulate cortex of patients with unipolar and bipolar depression

Authors: *J. A. AZEVEDO[1], B. S. CARTER[3], F. MENG[2], D. L. TURNER[2], M. DAI[2], A. F. SCHATZBERG[4], J. D. BARCHAS[5], E. G. JONES[6], W. E. BUNNEY[7], R. M. MYERS[8], H. AKIL[2], S. J. WATSON[2], R. C. THOMPSON[2];
[1]PiBS Neurosci., [2]Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI; [3]Whitehead Inst. for Biomed. Res., MIT, Cambridge, MA; [4]Dept. of Psychiatry and Behavioral Services, Stanford Univ., Palo Alto, CA; [5]Dept. of Psychiatry, Cornell Univ., Ithaca, NY; [6]Ctr. for Neurosci., Univ. of California - Davis, Davis, CA; [7]Psychiatry and Human Behavior, Univ. of California - Irvine, Irvine, CA; [8]HudsonAlpha Inst. for Biotech., Huntsville, AL

Abstract: MicroRNAs are a class of small, non-coding RNAs that canonically act as post-transcriptional regulators of gene expression. Given that microRNAs (miRNA) are heavily

enriched in the CNS and regulate many CNS processes, they have garnered intense interest, particularly in their relevance to CNS disorders. Multiple miRNAs have been shown to vary as a function of psychiatric disease state in several psychiatrically-relevant brain regions. However, these microRNAs have not been examined in any psychiatric disease state in the anterior cingulate cortex (AnCg), a brain region centrally involved in the regulation of mood and affect. Here we performed qPCR expression analyses of 29 miRNAs previously implicated in psychiatric illness (e.g. MDD, BP and/or schizophrenia) in AnCg of patients with MDD and BP versus control patients. Five microRNAs were differentially expressed in disease states: three in the BP cohort, one in the MDD cohort, and one across both states. In silico target prediction algorithms were used to identify putative targets of differentially expressed miRNAs and three mRNA candidates were selected based upon their previously published relevance to psychiatric illness. Luciferase reporter assays employing 3' UTRs of these candidate mRNAs were used to query miRNA/mRNA interactions. Site-directed mutagenesis of the putative seed domains alleviated each miRNA repression, validating these direct miRNA/mRNA interactions. Following target validations qPCR analyses were performed to determine whether changes in miRNA levels were correlated with changes in steady-state mRNA levels of these validated targets. Of the three mRNAs examined, one mRNA was overexpressed in our BP cohort, one was repressed in our MDD cohort, and one was unchanged in either disease state. In sum, this is the first study to demonstrate miRNA changes in AnCg as a function of psychiatric disease and 2) validate a miRNA as differentially expressed in both MDD and BP cohorts. Taken together, these findings support a mechanistic role for miRNAs in gene expression changes previously observed in psychiatric illness.

Disclosures: **J.A. Azevedo:** None. **B.S. Carter:** None. **F. Meng:** None. **D.L. Turner:** None. **M. Dai:** None. **A.F. Schatzberg:** None. **J.D. Barchas:** None. **E.G. Jones:** None. **W.E. Bunney:** None. **R.M. Myers:** None. **H. Akil:** None. **S.J. Watson:** None. **R.C. Thompson:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.09/I12

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Pritzker Neuropsychiatric Disorder Research Consortium

NIH grant: R01MH104261

ONR grant: N00014-12-1-0366

Hope for Depression Research Foundation

Title: Polyamine metabolic enzyme gene expression analysis of human amygdala of major depressive disorder samples

Authors: *V. SHARMA[1], M. HAGENAUER[1], S. CHAUDHURY[1], R. C. THOMPSON[1], R. M. MYERS[2], A. F. SCHATZBERG[3], J. D. BARCHAS[4], W. E. BUNNEY[5], H. AKIL[1], S. J. WATSON[1];
[1]Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI; [2]Hudsonalpha Inst. for Biotech., Huntsville, AL; [3]Dept. of Psychiatry, Stanford Sch. of Med., Stanford, CA; [4]Dept. of Psychiatry, Weil Cornell Med. Col., New York, NY; [5]Dept. of Psychiatry, Univ. of California Irvine, Irvine, CA

Abstract: The polyamine pathways are important in cell growth as well as proliferation and are activated (or regulated) by G-proteins, protein kinases, nucleotide cyclases and receptors. Few recent studies have focused on the role of polyamines and their metabolic enzymes in the etiology and pathology of mental disorders. Altered levels of mRNA of polyamine enzymes arginase II (ARG2), S-adenosylmethionine decarboxylase (AMD1), and antizymes 1 and 2 (OAZ1 and OAZ2) have been observed in conditions like schizophrenia, major depressive disorder and bipolar disorder. The amygdala plays an important role in emotion, motivation and other higher cognitive functions which result in the addition of the subjective value to the stimulus. It appears to act as a neural gateway for binding the sensory representations with the neural correlates of emotional and motivational valence, thereby linking sensory information to adaptive responses and meaningful experiences. Because the human amygdala is a heterogeneous structure containing numerous nuclei that vary in size and shape through the anterior-posterior axis, we utilized laser capture microdissection to precisely dissect the nuclei from the human amygdala into ten divisions (Lateral (L), Basal (B), Accessory Basal (AB), Central (CE), Medial (M), Cortical (CO), Periamygdaloid Cortex (PAC), Amygdalohippocampal Area (AHA), Anterior Amygdaloid Area (AAA) and Paralaminar (PL) nuclei) followed by microarray gene expression analysis. Microarray data revealed the altered levels of the above said genes in the different sub-nuclei of amygdala which prompted us to further investigate the genes for polyamine metabolism. Thus, in the present study we assess the levels of gene expression of polyamine metabolic enzymes in various amygdala subnuclei of postmortem human brain of normal healthy and major depressive disorder subjects. The findings will help us in understanding the role of polyamines and their metabolic enzymes in the functioning of human amygdalar sub-nuclei in major depressive disorder.

Disclosures: V. Sharma: None. **M. Hagenauer:** None. **S. Chaudhury:** None. **R.C. Thompson:** None. **R.M. Myers:** None. **A.F. Schatzberg:** None. **J.D. Barchas:** None. **W.E. Bunney:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.10/I13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH53327

NIH Grant MH064673

NIH Grant MH066392

Title: Expression of GABA(A) receptor trafficking molecules in schizophrenia brain

Authors: *T. M. MUELLER[1], V. HAROUTUNIAN[2], J. H. MEADOR-WOODRUFF[1]; [1]UAB, Birmingham, AL; [2]Mt Sinai Sch. of Med., Bronx, NY

Abstract: Schizophrenia is a complex neuropsychiatric illness of which the exact mechanisms remain unclear. Our lab has previously identified abnormalities of the gene and protein expression of some receptor subunits and associated trafficking molecules, disrupted N-glycosylation, and differences in subunit localization of glutamate receptor subunits. More recently, we have expanded these studies to determine if other neurotransmitter systems, such as the GABAergic system, exhibit similar alterations. We have previously measured the protein expression of GABA(A) receptor (GABAAR) subunits in postmortem human superior temporal gyrus (STG) between schizophrenia and comparison subjects, finding no difference between diagnostic groups. However, abnormalities of GABAAR subunit N-glycosylation of the α1, β1, and β2 subunits and atypical subcellular distribution of GABAAR β1 and β2 subunit isoforms have been identified in schizophrenia STG. Similar to the glutamatergic system, GABAARs must interact with a variety of other proteins in order to be correctly trafficked from the endoplasmic reticulum to the synapse. Our current study measures the expression of molecules necessary for the proper forward trafficking of GABAARs to determine if other factors, in addition to abnormal N-glycosylation, may contribute to the disrupted subcellular localization of GABAAR subunits in schizophrenia. To inform this question, we first measured the gene and protein expression of several molecules known to be necessary for GABA receptor trafficking in total STG homogenates and found reduced transcription of GABA receptor associated protein (GABARAP), gephyrin, and brefeldin A-inhibited guanine nucleotide exchange factor (ARFGEF2) mRNA in schizophrenia; however, we did not find any corresponding alteration in the relative abundance GABARAP or gephyrin protein. Furthermore, when assessed in a synapse-enriched subcellular fraction, gephyrin was found to be normally expressed in schizophrenia brain. These data demonstrate that protein levels of the trafficking molecules under investigation are not disrupted in schizophrenia STG. However, since N-glycosylation can play an important role in mediating protein-protein interactions by directly interacting with glycan binding proteins or by exerting steric effects affecting core protein conformation and/or binding site accessibility, the possibility remains that abnormalities of N-glycosylation may disrupt the integrity of protein-protein interactions between these molecules and intact GABAARs, thereby contributing to abnormal GABAAR subunit subcellular localization.

Disclosures: **T.M. Mueller:** None. **V. Haroutunian:** None. **J.H. Meador-Woodruff:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.11/I14

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Vahlteich Award

Title: GABAergic gene expression in the anterior cingulate cortex in schizophrenia

Authors: ***G. C. BRISTOW**[1], M. S. SODHI[2];
[1]Dept. of Pharm. Practice and Ctr. for Pharmaceut. Biotech., [2]Dept. of Pharm. Practice and Ctr. for Pharmaceut. Biotechnology, Dept. of Psychiatry, Univ. of Illinois At Chicago, Chicago, IL

Abstract: GABAergic dysfunction has been strongly implicated in the pathophysiology of schizophrenia. We have previously shown that sex differences in the expression of GABAA receptor subunits occur in the anterior cingulate cortex (ACC) in schizophrenia. These analyses revealed that in male groups, the expression of GABAergic genes was generally lower in schizophrenia cases compared to the controls, with significantly lower expression levels of GABAAα5, GABAAβ1, and GABAAε. In females, the expression of GABAergic genes was higher in the schizophrenia cases, with significantly higher expression of the GABAAβ1 and GAD67 genes. Analyses of the effects of medication in the male schizophrenia subjects revealed significantly higher expression of GABAAα1-3, GABAAβ2, GABAAγ2, and GAD67 in the medicated group compared to the unmedicated group, indicating that anti-psychotic treatment may have "corrected" the deficits in GABAergic gene expression observed. Investigations of the GABA transporter genes have not been reported in the ACC in schizophrenia. Here we have extended our study to analyse the expression of a gamma-aminobutyric acid transporter GAT2 in order to further examine the regulation of GABAergic neurotransmission in the ACC in schizophrenia. We have tested the ACC of post-mortem subjects with schizophrenia (n=21) and a comparison group of individuals without a history of psychiatric illness (n=18). Our data indicate that the expression of GAT2 does not differ in schizophrenia relative to controls, or between male and female groups. GAT2 expression is also not altered by medication status. In summary, our data indicate that there are sex differences in the expression of several GABAA receptor genes and GAD67, but not GAT2, in the ACC in schizophrenia. The authors thank Vahram Haroutunian, Director of the Alzheimer's disease and Schizophrenia Brain Bank, for post-mortem tissue, and John A. Bostrom for technical assistance. Project funded by the Vahlteich Award to MS.

Disclosures: **G.C. Bristow:** None. **M.S. Sodhi:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.12/I15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH grant MH074016

NIH grant MH087752

NIH grant MH094445

Doris Duke Clinical Scientist Award (REM)

Lindsay Brinkmeyer Schizophrenia Research Fund

Title: Region and pyramidal cell level expression of glutamate transporters EAAT1 and EAAT2 and their splice variants in schizophrenia

Authors: *S. M. O'DONOVAN[1], K. HASSELFELD[1], D. BAUER[2], M. SIMMONS[3], P. ROUSSOS[4], V. HAROUTUNIAN[4], J. H. MEADOR WOODRUFF[3], R. E. MCCULLUMSMITH[1];
[1]Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH; [2]Wellsley Col., Wellsley, MA; [3]Univ. of Alabama, Birmingham, AL; [4]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Dysregulation of the glutamate transporters EAAT1 and EAAT2 and their isoforms have been implicated in schizophrenia. EAAT1 and EAAT2 expression has been studied in different brain regions but the prevalence of astrocytic glutamate transporter expression masks the more subtle changes in EAAT isoforms in neurons in the cortex. The mRNA levels of EAAT1, EAAT2 and the splice variants EAAT1 exon9skipping, EAAT2 exon9skipping and EAAT2b were analyzed by RT-PCR in an enriched population of pyramidal neurons. Region-level expression of these transcripts was measured in postmortem schizophrenia (n=25) and controls (n=25) in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex. Using laser capture microdissection, pyramidal neurons were cut from the anterior cingulate cortex of postmortem schizophrenia (n=20) and control (n=20) subjects. The relationship between selected EAAT polymorphisms and EAAT splice variant expression was also explored. There were no changes in EAAT isoform mRNA levels in the DLPFC in schizophrenia compared to control subjects. EAAT2 exon9skipping mRNA was increased ($p<0.05$; 38%) at region-level in the anterior cingulate cortex with no significant change in other EAAT variants at region-level. Anterior cingulate cortex pyramidal cell expression of EAAT2b mRNA was significantly increased ($p<0.001$; 67%) in schizophrenia subjects compared to controls. There was no significant change in other EAAT variants. EAAT2 SNPs were significantly associated

with changes in EAAT2 isoform expression. To control for possible medication effects, rats were administered a chronic course (9 months) of haloperidol-decanoate, a typical antipsychotic drug. Neuronal EAAT2b mRNA levels were not significantly altered in these animals. The novel finding that EAAT2b levels are increased in populations of anterior cingulate cortex pyramidal cells further demonstrates a role for neuronal glutamate transporter splice variant expression in schizophrenia.

Disclosures: S.M. O'Donovan: None. **K. Hasselfeld:** None. **D. Bauer:** None. **M. Simmons:** None. **P. Roussos:** None. **V. Haroutunian:** None. **J.H. Meador Woodruff:** None. **R.E. McCullumsmith:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.13/I16

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH094445

Title: Altered expression of adenosine system components in pyramidal neuron and glial cell populations in schizophrenia

Authors: *K. A. HASSELFELD[1], R. H. KOENE[1], S. M. O'DONOVAN[1], R. C. ROBERTS[2], R. E. MCCULLUMSMITH[1];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Schizophrenia is a debilitating and complex neuropsychiatric disorder that affects approximately 1.0% of the world's population. A leading hypothesis of the etiology of schizophrenia is a hypofunctioning glutamatergic system, which has been shown to produce negative and cognitive symptoms. The neuromodulator adenosine regulates glutamate transmission through its effects on neurons, both postsynaptically and presynaptically, and glial cells. We hypothesize that the adenosine system is differentially regulated in enriched populations of pyramidal neurons and glial cells in schizophrenia. The expression of adenosine pathway components were measured at region-level and in enriched populations of pyramidal neurons and astrocytes cut by laser capture microdissection from the dorsal lateral prefrontal cortex (DLPFC) of postmortem schizophrenia (n=10) and control (n=10) subjects. QPCR was used to analyze mRNA levels of adenosine 2a receptor (A2aR), adenosine deaminase (ADA), adenosine kinase (ADK), ectonucleoside triphosphate diphosphohydrodase 1 and 2 (ENTPD1/2), ecto 5'-nucleotidase (NT5E) and equilibrative nucleoside transporter 1 (ENT1). At the region-level in the DLPFC, ADA was increased ($p<0.05$; 60%) in schizophrenia while ENTPD1 mRNA levels were significantly decreased ($p<0.05$; 39%). There was no significant change in the other

transcripts at the region level. Expression of ADA mRNA was also increased in pyramidal neurons ($p<0.05$; 73%). ENT1 was significantly decreased ($p<0.05$; 29%) in pyramidal neurons in schizophrenia, while the other transcripts were not changed. ENTPD1 mRNA levels were significantly decreased ($p<0.05$; 37%) in astrocytes. There were no other significant changes between schizophrenia and control subjects at the astrocyte cell level. Components of the adenosine pathway were differentially expressed among control and schizophrenia subjects at region level and in both pyramidal neuron and astrocyte cell populations. This suggests an impairment of the adenosine system consistent with a loss of capacity to transport nucleosides and changes in adenosine metabolism.

Disclosures: K.A. Hasselfeld: None. **R.H. Koene:** None. **S.M. O'Donovan:** None. **R.C. Roberts:** None. **R.E. McCullumsmith:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.14/I17

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH074016

NIH Grant MH087752

NIH Grant MH094445

Duke Clinical Scientist Award

Lindsay Brinkmeyer Schizophrenia Research Fund

Title: Expression of EAAT mRNA isoforms in Nissl-stained astrocyte populations in post-mortem schizophrenia

Authors: *R. KOENE[1], K. HASSELFELD[1], S. M. O'DONOVAN[1], R. C. ROBERTS[2], R. E. MCCULLUMSMITH[1];
[1]Dept. of Neurosci. and Behavioral Psychiatry, Univ. of Cincinnati, Cincinnati, OH; [2]Dept. of Psychiatry and Behavioral Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Schizophrenia is a debilitating neurological disorder which has been linked to abnormalities in glutamatergic function in the brain. The main function of excitatory amino acid transporters (EAATs) is the reuptake and removal of glutamate from synapses. Astroglial transporters account for up to 90% of transport of glutamate in the forebrain. Astrocytes express high levels of EAATs as well as of glial fibrillary acidic protein (GFAP). We propose EAAT expression is decreased in schizophrenia. 14µm dorsolateral lateral prefrontal cortex (DLPFC)

sections were labelled with GFAP, an astrocyte marker, or stained with Nissl stain, which stains all nucleic acids (n=3 subjects/group). Astrocytes were then cut and captured by laser capture microdissection (LCM) based on visualization of GFAP labelled cells or cell morphology (Nissl). Gene expression of GFAP (astrocyte marker) was measured using QPCR in the enriched populations of astrocytes. Nissl-stained astrocytes had significantly higher expression of GFAP than GFAP-labelled astrocytes (t-test; $p < 0.01$). Based on these results, 14µm thick DLPFC tissue sections from control (n=10) and schizophrenia (n=10) subjects were stained with Nissl and an enriched population of astrocytes were captured. Gene expression levels of EAAT1, EAAT2 and their isoforms EAAT1 exon9 skipping, EAAT2 exon9 skipping , EAAT2b and EAAT2 intron7 retaining were measured using QPCR. EAAT1, EAAT2, EAAT1x9 skipping and EAAT2x9 skipping astrocytic mRNA expression was decreased 25-30% in schizophrenia subjects compared to controls. EAAT2intron7 retaining astrocytic mRNA decreased by 35% in schizophrenia subjects, and EAAT2b expression in schizophrenia was significantly decreased (44% compared to controls; t-test, $p \leq 0.05$). Astrocytes, identified by morphology using a Nissl staining protocol, had higher levels of GFAP expression, validating our astrocyte identification and LCM capture protocol. As a result, we applied this protocol to measure EAAT isoform mRNA expression levels in astrocytes in schizophrenia. This cell-specific approach found substantial decreases in EAAT levels, with EAAT2b expression being significantly decreased. These results support previous findings that show alteration in the glutamate reuptake system in schizophrenia.

Disclosures: R. Koene: None. **K. Hasselfeld:** None. **S.M. O'Donovan:** None. **R.C. Roberts:** None. **R.E. McCullumsmith:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.15/I18

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH094445

Title: Altered expression of monocarboxylate transporter 1 in laser captured pyramidal neurons and astrocytes in schizophrenia

Authors: *C. R. SULLIVAN, S. O'DONOVAN, R. KOENE, K. HASSELFELD, R. MCCULLUMSMITH;
Psychiatry, Univ. of Cincinnati, Cincinnati, OH

Abstract: Schizophrenia is a complex and disabling illness characterized by impairments in attention, cognition, planning, and social function. There is evidence that abnormal lactate

metabolism and mitochondrial dysfunction might contribute to these deficiencies. In normal brain, lactate is rapidly synthesized from pyruvate in astrocytes and can be shuttled to neighboring cells via monocarboxylate transporters (MCTs). MCTs on neurons rapidly transport lactate into the neuron, where it is converted into pyruvate by LDH, which may enter the TCA cycle to generate ATP, suggesting a critical role for lactate in neuronal transmission. We hypothesize that lactate metabolism is differentially regulated in enriched populations of pyramidal neurons and astrocytes in schizophrenia. Enriched populations of pyramidal neurons and astrocytes were cut by laser capture microdissection from the dorsal lateral prefrontal cortex (DLPFC) in subjects with schizophrenia (n=10) and control (n=10) subjects. QPCR was used to analyze cell and region level expression of mRNAs for MCT1, MCT2, and MCT4. At the region level, MCT1, but not MCT2 or MCT4, mRNA was increased (p=0.00997) in subjects with schizophrenia compared to controls. MCT1 mRNA was also increased in pyramidal neurons (p=0.01298; 49%) and in astrocytes (p=0.02620; 39%) in subjects with schizophrenia compared to controls. There was no significant difference in MCT2 or MCT4 transcripts in pyramidal neurons or astrocytes. MCT1 transporters in the lactate shuttle pathway were differentially expressed among control and schizophrenia subjects at region level and in both Pyramidal neuron and astrocyte cell populations. A global increase in MCT1 transcripts might suggest an initial compensatory mechanism for decreased lactate production, inefficient lactate shuttling to neurons, or low transporter activity in schizophrenia. In conclusion, lactate metabolism is altered in schizophrenia by cell-type dependent changes possibly contributing to abnormal neuronal transmission.

Disclosures: C.R. Sullivan: None. **S. O'Donovan:** None. **R. Koene:** None. **K. Hasselfeld:** None. **R. McCullumsmith:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.16/I19

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH094445

Title: Analysis of the EAAT2 interactome coupling to metabolic function in schizophrenia

Authors: *A. E. GARDNER, S. M. O'DONOVAN, K. E. CLICK, A. J. FUNK, R. E. MCCULLUMSMITH;
Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Schizophrenia is an often devastating neurodevelopmental illness characterized by positive, negative, and cognitive deficits that contribute to profound impairment in the afflicted.

We have previously reported changes in glutamate transporter expression in this illness, extending the NMDA receptor hypofunction hypothesis beyond the NMDA receptor. Our findings suggest a profound deficit in glutamate reuptake in multiple brain regions that may contribute to the deficits of neuroplasticity found in this illness. The excitatory amino acid transporter 2 (EAAT2) has a rich protein-protein interactome, which consists of a myriad of structural, signaling, and metabolic proteins. The function of this protein complex has a central role in the maintenance of sodium and potassium gradients, the production of energy intermediates including lactate and pyruvate, as well as facilitation of the glutamate/glutamine cycle. We hypothesize that the integrity of this protein complex is compromised in schizophrenia. We have developed an affinity purification-LC/MSMS protocol to isolate, characterize, and quantify the EAAT2 interactome in human postmortem brain tissues. Data will be presented from subjects with schizophrenia and a control group for key elements of the EAAT2 interactome. We will use a well-characterized bioinformatics workflow to normalize our data across subjects and quantify changes in the protein content between diagnostic groups. We anticipate that there are profound changes in the metabolic coupling of EAAT2 to metabolic function. We predict that changes in the EAAT2 interactome will reflect uncoupling of EAAT2 to mitochondrial function, with decreases in metabolic enzymes and increases in cytoplasmic proteins associated with trafficking away from the perisynaptic microdomain.

Disclosures: A.E. Gardner: None. **S.M. O'Donovan:** None. **K.E. Click:** None. **A.J. Funk:** None. **R.E. McCullumsmith:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.17/I20

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH076060

Life Technologies

Title: Developmental molecular profiles of human choroid plexus epithelial cells determined by whole transcriptome rna sequencing

Authors: K. ATHANAS[1], E. KOHLBRENNER[1], S. JACKSON[2], K. VARMA[2], S. MAUNEY[1], K.-C. SONNTAG[3], S. BERRETTA[4], *T.-U. WOO[5];
[1]Cell. Neuropathology, McLean Hosp., Belmont, MA; [2]Genet. Analysis, Life Technologies, San Francisco, CA; [4]Lab. of Translational Neurosci., [5]Lab. of Cell. Neuropathology, [3]McLean Hosp/Harvard Med. Sch., Belmont, MA

Abstract: Increasing evidence from rodent studies has implicated the homeobox protein orthodenticle homeobox 2 (OTX2) as playing a critical role in orchestrating the postnatal maturation of neural circuitry of the cerebral cortex. In addition, it appears that the choroid plexus represents a major supply of OTX2 in the postnatal brain. In this study, we use whole transcriptome RNA sequencing (RNA-Seq) to determine the molecular profiles associated with human choroid plexus epithelial cells during postnatal development. Choroid plexus epithelial cells are obtained using laser capture microdissection (LCM). RNA is extracted from LCM caps using the Arcturus Picopure RNA purification kit. Libraries are constructed using the Ion Ampliseq Human Transcriptome kit, templated using Ion Chef, and sequenced on Ion Torrent Proton instruments using PI chips. Sequences are aligned using Ion Torrent Suite algorithms. Transcript abundance is analyzed by normalizing to reads per million reads using the Ampliseq RNA analysis plug-in available in the Torrent Suite software. Findings of this study will provide insight into the molecular underpinnings of the regulation of OTX2 production in the choroid plexus during postnatal human development. In this context, OTX2 deficit has recently been observed in subjects with schizophrenia and developmental events that are regulated by OTX2 also appear to be disturbed in this illness. As such, comparison of findings of this study and possible alteration in the molecular signature of choroid plexus epithelial cells in schizophrenia may shed light onto the pathophysiology of this illness and potential corrective strategies.

Disclosures: K. Athanas: None. **E. Kohlbrenner:** None. **S. Jackson:** None. **K. Varma:** None. **S. Mauney:** None. **K. Sonntag:** None. **S. Berretta:** None. **T. Woo:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.18/I21

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH076060

MH080272

Title: Prefrontal cortical perineuronal net ensheathment of pyramidal neurons in schizophrenia

Authors: *K. M. ATHANAS[1], T.-U. W. WOO[1,2,3];
[1]Lab. for Cell. and Mol. Neuropathology, McLean Hosp., Belmont, MA; [2]Dept. of Psychiatry, Beth Israel Deaconess Med. Ctr., Boston, MA; [3]Dept. of Psychiatry, Harvard Med. Sch., Boston, MA

Abstract: Schizophrenia (SZ) is a debilitating psychotic disorder that typically onsets during late adolescence or early adulthood. Perineuronal nets (PNNs) are extracellular aggregates predominately composed of chondroitin sulfate proteoglycan (CSPG), tenascin-R, link proteins,

and hyaluronan that ensheath the somata and dendritic branchings of many neurons, including pyramidal neurons and the inhibitory neurons that contain parvalbumin (PV). We have recently found that the density of neurons ensheathed by PNNs appears to be significantly decreased in the prefrontal cortex (PFC) in SZ, but in that study we did not distinguish the specific cell types that might be involved. Here, we test the hypothesis that the ensheathment of pyramidal neurons by PNNs is deficient in SZ. In a cohort of SZ and 20 demographically matched controls (C) subjects, we use biotin-labeled lectin from Wisteria floribunda aggultinin (WFA) to histochemically visualize CSPG-containing PNNs in post mortem tissue of the PFC, followed by Nissl staining. Pyramidal neurons are identified based on morphological characteristics. In a blind fashion, we quantify the densities of pyramidal cells that are surrounded by and devoid of PNNs. PNNs are known to regulate neural circuitry formation, structural plasticity, synaptic maturation and stabilization, all of which are processes thought to be disrupted in SZ. Findings of this study will shed light onto the cell type-specific nature of PNN deficit in SZ and will provide insight into the pathophysiology of dysconnectivity of cortical excitatory circuits.

Disclosures: K.M. Athanas: None. **T.W. Woo:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.19/I22

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH MH076060

NIH MH080272

Title: UBE3B expression in the prefrontal cortex during development and in schizophrenia

Authors: *E. KOHLBRENNER[1], W. T.-U. WOO[2,3,4];
[2]Lab. for Cell. and Mol. Neuropathology, [1]McLean Hospital; Harvard Med. Sch., Belmont, MA; [3]Dept. of Psychiatry, Beth Israel Deaconess Med. Ctr., Boston, MA; [4]Dept. of Psychiatry, Harvard Med. Sch., Boston, MA

Abstract: The prefrontal cortex, a major site of dysfunction in schizophrenia, undergoes extensive developmental changes during adolescence, including the remodeling and pruning of circuitry in neuronal networks that eventually lead to enhanced cognitive decision making ability in adulthood. In most cases of schizophrenia, the manifestation of symptoms does not begin until late adolescence and early adulthood, suggesting that developmental events in the peri-adolescent period could contribute to the onset of this disorder. Our previous work explores gene expression regulation in normal, postnatal human development; specifically at layer 3 pyramidal cells that furnish corticocortical connections in the prefrontal cortex. We identified genes that are

both developmentally regulated and differentially expressed in schizophrenia. One of these genes is the ubiquitin ligase-encoding gene, UBE3B, a paralog to the widely studied UBE3A which has been previously correlated with autistic behaviors in mice. Ubiquitination influences cellular signaling cascades by posttranslational protein modification and regulates neuronal migration, neurogenesis, synaptic elimination and formation as a result. Alterations in the ubiquitination system are known to contribute to developmental neurological diseases such as Angelman Syndrome and other autism spectrum disorders. Previous protein and genetic expression analysis findings suggest that UBE3B expression increases developmentally and is decreased in schizophrenic samples, and thereby could contribute to the developmental pathophysiology of schizophrenia by disturbing periadolescent synaptic refinement of prefrontal cortical circuitry. We compared a cohort of schizophrenia versus control human post-mortem prefrontal cortex (Brodmann's area 9) (N=30 and 15, respectively), immunohistochemically stained and quantified in a blind fashion with an anti-ube3b polyclonal antibody and Nissl staining, to test the hypothesis that the density of UBE3B-expressing cells is decreased in schizophrenia. Preliminary results of this ongoing investigation indicate that schizophrenic samples have a lower percentage of ube3b-expressing cells, as also seen in our previous genetic analysis. Findings of this study will deepen our current understanding of the molecular pathophysiology of schizophrenia and its onset.

Disclosures: E. Kohlbrenner: None. **W.T. Woo:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.20/I23

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Intramural

Title: Whole-genome expression analysis across major mental disorders allows examining disease-specific changes and potential common molecular pathogenic mechanisms

Authors: *R. KRAMER[1], M. MISTRY[2], M. FROMER[3,4], A. ELKAHLOUN[4,2], N. FENG[1], B. K. LIPSKA[1,3];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]HSPH Bioinformatics Core, Harvard Sch. of Publ. Hlth., Boston, MA; [3]Dept. of Genet. and Genomic Sci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Natl. Human Genome Inst., Bethesda, MD

Abstract: Methods: We assessed transcription alterations in schizophrenia (SZ), bipolar disorder (BP) and major depression (MDD) as compared to controls (C) in hippocampus, dorsolateral prefrontal cortex (DLPFC) and dura in a large cohort of individuals (total number 470 subjects

for hippocampus, 691 for DLPFC and 150 for dura). RNA was extracted using RNAeasy Qiagen kits. Biotin labeled amplified RNAs were obtained with Affymetrix 3'IVT express kits and hybridized to the Illumina HumanHT-12_V4 Beadchips. The data were imported into R using the BeadArray package. VST and quantile normalization were performed. ComBat was used to remove batch effects. SVA was used for finding latent covariates. Quantitative PCR was used for validation of results in a large cohort of samples (~700). Results: Overall, not many genes were significantly different between controls and diagnostic groups (N=203, P <.05), and the fold changes were small (1%-18%). There were more DE genes in SZ than BP in DLPFC (N=138 and N=13 respectively), and vice versa in Hippocampus (N=16 and N=38 respectively). There were no differentially expressed genes in SZ vs C in dura. Variation was primarily associated with Diagnosis and not with confounders (pH, RIN, race, sex). More genes (60%) were downregulated in SZ in DLPFC than upregulated. ALDH1a1, known to be involved in dopamine metabolism, was validated by qPCR in DLPFC and Hippocampus. Conclusions: Data generated from postmortem brain tissue is a valuable resource for understanding the molecular nature of psychiatric disorders, and the validated gene, ALDH1A1, has potential clinical relevance due to its known role in dopamine metabolism.

Disclosures: R. Kramer: None. **M. Mistry:** None. **M. Fromer:** None. **A. Elkahloun:** None. **N. Feng:** None. **B.K. Lipska:** None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.21/I24

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: ROI-MH 086522

Title: Decreased number of perineuronal nets in the reticular thalamic nucleus of individuals with schizophrenia and bi-polar disorder

Authors: *M. ARDELT;
McLean Hosp., Belmont, MA

Abstract: Background: Growing evidence shows that neuronal circuits linking the prefrontal cortex to the mediodorsal nucleus of the thalamus are disrupted in subjects with schizophrenia (SZ). The reticular nucleus of the thalamus (RTN) receives massive cortical projections and is connected to the mediodorsal and other thalamic nuclei; it is thus in a strategic position to gate the flow of cortico-thalamic information. A large number of RTN neurons are enveloped by perineuronal nets (PNNs), specialized extracellular matrix structures shown to regulate synaptic functions. Growing evidence shows that PNNs are markedly decreased in several brain regions

in SZ, including the medial temporal lobe and prefrontal cortex. Similar decreases in the RTN are likely to disrupt synaptic connectivity in SZ, thus impacting the information flow within cortico-thalamic circuits. With the present studies, we tested the hypothesis that PNNs in the RTN may be decreased in subjects with SZ. **Methods**: To visualize PNNs in serial sections which contained the thalamus from healthy controls (n=15), SZ (n=15) and bipolar disorder (BD; n=15) sections were labeled using *wisteria floribunda* agglutinin lectin (WFA). Stereology-based computer-assisted light microscopy was used to estimate numerical densities of PNNs. **Results**: Step-wise regression analysis shows a significant reduction of numerical densities of WFA-positive PNNs in the RTN of subjects with SZ ($p=0.010$; adjusted for antipsychotic exposure within the last six months of life) as well as in subjects with BD ($p=0.012$; adjusted for lifetime exposure to lithium) as compared to healthy controls. **Conclusions:** Our results show significant decreases in PNN number in the RTN of subjects with SZ and also BD. Such decreases may impact synaptic stability and regulation of glutamatergic inputs onto inhibitory RTN neurons, in turn disrupting thalamic connectivity. Parallel decreases of WFA-positive PNNs in SZ and BD were unexpected and raise the possibility of overlapping pathological findings in the RTN of subjects with major psychoses.

Disclosures: M. Ardelt: None.

Poster

312. Major Mental Disorders: Human Postmortem Studies

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 312.22/I25

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH098554

Title: CRH, CRH-R1, CRH-R2 and CRH-BP in the prefrontal cortex and hippocampus of teenage suicide victims

Authors: *X. REN, H. RIZAVI, G. N. PANDEY;
Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Suicide is a major public health concern, and in youth, it is the second most frequent cause of death in the United States. Although the neurobiology of adult suicide has been studied, the neurobiology of teenage suicide has been less studied. Recent studies suggest that there is a disturbance in hypothalamic -pituitary-adrenal (HPA) axis in depression and suicide. In order to determine the role of CRH, CRH-R and CRH-BP in teenage suicide victims, we studied CRH, CRH-R1, CRH-R2 and CRH-BP levels in the prefrontal cortex (PFC) and hippocampus of postmortem teenage suicide victims compared and control subjects. The protein expression of CRH, CRH-R1, CRH-R2 and CRH-BP was determined in the prefrontal cortex (PFC),

Brodmann area 9 (BA-9) and hippocampus obtained from 17 teenage (13-19 years) suicide victims and 17 control subjects by Western blot technique. Postmortem brain samples were obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore, Maryland. We found that the protein expression level of CRH was significantly increased, while the protein expression levels of CRH-R1, CRH-R2 and CRH-BP were significantly decreased in the PFC of teenage suicide victims compared with control subjects. However, there was no difference in the protein expression level of CRH, CRH-R1, CRH-R2 and CRH-BP in the hippocampus of teenage suicide victims compared to control subjects. These results suggest that the alterations of CRH, CRH-R1, CRH-R2 and CRH-BP in the PFC of teenage suicide victims may be related to the pathophysiology of teenage suicide. These results also suggest region-specific alterations of protein levels of CRH, CRH-R1, CRH-R2 and CRH-BP.

Disclosures: X. Ren: None. **H. Rizavi:** None. **G.N. Pandey:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.01/I26

Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA036487-01

Title: The effects of smoking reduced nicotine cigarettes upon resting state functional connectivity, craving and withdrawal in young smokers

Authors: *P. FAULKNER, D. GHAHREMANI, C. COX, G. HELLEMANN, E. LONDON; Semel Inst. for Neurosci. and Human Behaviour, UCLA, Los Angeles, CA

Abstract: Smoking contributes to more than 540,000 premature deaths each year in the United States, and enacting a policy to reduce the nicotine content is viewed as a way to reduce cigarette use. This study examined whether the nicotine yield of a cigarette affects the response to smoking, measured as functional coupling between brain networks that have been shown to be related to cigarette craving, withdrawal and cognition - the executive control network (ECN), default mode network (DMN), and the salience network (SN) - along with behavioural measures of craving, withdrawal, and sustained attention. Fifteen participants (11 men, 4 women), 18-25 years of age, who smoked cigarettes daily, each completed testing on 5 days in two sessions: before and after the first cigarette of the day (after overnight abstinence). Testing included resting state fMRI scans, questionnaire measures including the Urge To Smoke (UTS) scale and the Shiffman-Jarvik Withdrawal (SJW) scale, and the Rapid Visual Information Processing (RVIP) task, a test of sustained attention. Participants smoked a research cigarette containing one

of four nicotine yields (0.027, 0.110, 0.231 or 0.763 mg), or their own preferred-brand. Independent Component Analyses of the fMRI data revealed 3 ECN networks, 5 DMN networks, and 1 SN network. Positive coupling between ECN and DMN networks and SN and DMN networks was reduced after smoking, with reduction in a greater number of networks after smoking the participants' preferred brand than the .027 mg cigarette. Smoking also enhanced sustained attention, as measured via RVIP hits and A' scores, with greater improvements in A' scores after smoking cigarettes with higher nicotine yields. Finally, smoking reduced cigarette craving and withdrawal, irrespective of the type of cigarette smoked or the nicotine yield. These results extend previous observations that both ECN-DMN coupling and SN-DMN coupling are reduced following cigarette smoking, by showing that these changes depend upon the nicotine yield of the cigarette. The results also suggest that improvements in sustained attention depend on the nicotine content of a cigarette, but that self-reported smoking-induced reductions in craving and withdrawal do not. The results suggest that RSFC may be a more sensitive index of response to smoking than subjective self-reports of craving.

Disclosures: P. Faulkner: None. **D. Ghahremani:** None. **C. Cox:** None. **G. Hellemann:** None. **E. London:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.02/I27

Topic: C.17. Drugs of Abuse and Addiction

Support: University of Houston-Clear Lake Faculty Research Support Fund

Title: Effect of gender and estrous cycle phase on nicotine withdrawal syndrome in the rat

Authors: *M. HENCEROTH, J. R. CAMPBELL, M. L. CANDELARIO, C. L. AGUILAR, C. A. MADISON, E. A. ODOM, M. F. MERIANO, D. H. MALIN;
Univ. of Houston Clear Lake, Houston, TX

Abstract: Several studies have suggested differences in smoking cessation withdrawal syndrome as a function of gender and estrous cycle phase. There is a need to create rodent models of these effects so that they can be studied in the laboratory. While there is a large literature on nicotine withdrawal syndrome in the rat, there are very few studies with female subjects. The probable reason for this is female rats' four-day estrous cycle, which may cause variability in study results. The present research used histological examination to determine the precise estrous phase at the time of testing. The subjects were 16 female rats and 9 male rats, all five to six months old. Vaginal fluid was collected with a micropipette, placed on a glass slide, and stained with methylene blue. The slides were examined under a microscope for phase-identifying cell

types. Once the recurring patterns of estrous phases had been identified, experiments were scheduled for either the proestrus phase (n = 8), representing a follicular portion of the cycle, and the metestrus phase (n = 8) representing a luteal portion. The predicted estrous phases were histologically confirmed immediately after each experiment. For seven days before each experiment, rats were continuously infused with 9 mg/kg/day s.c. nicotine bitartrate. On the seventh day, each subject was challenged with 1 mg/kg of the nicotinic antagonist mecamylamine. This dose has been confirmed to precipitate a vigorous withdrawal syndrome in nicotine dependent rats only. Subjects were observed over 30 min. on a standard checklist of somatically expressed withdrawal behaviors. Male rats displayed 27.7 ± 3.4 withdrawal signs (M ± SEM), while female rats in their proestrus phase exhibited an almost identical 28.5 ± 2.8 signs (M ± SEM). Female rats in metestrus displayed 42.3 ± 5.1 signs (M ± SEM). One-way ANOVA revealed a significant difference among groups, p = .024. Post-hoc comparisons (Fisher's LSD test) revealed significant differences between metestrus females and proestrus females, p = .022 and between metestrus females and males, p = .013. There was no significant difference in withdrawal signs between proestrus females and males, p = .879. This confirms the interaction between gender and estrus cycle in modulating nicotine dependence and withdrawal. It also provides a laboratory model to study the biological basis and experimental treatment of this phenomenon.

Disclosures: **M. Henceroth:** None. **J.R. Campbell:** None. **M.L. Candelario:** None. **C.L. Aguilar:** None. **C.A. Madison:** None. **E.A. Odom:** None. **M.F. Meriano:** None. **D.H. Malin:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.03/I28

Topic: C.17. Drugs of Abuse and Addiction

Support: University of Houston-Clear Lake Faculty Research Support Fund

Title: A subtype-specific NPFF receptor antagonist reverses nicotine dependence

Authors: ***D. H. MALIN**[1], M. M. HENCEROTH-CHOMIAK[2], J. J. IZYGON[2], D. J. MCGHIEY[2], K. M. BRIGHT[2], D. M. NGHIEM[2], P. GOYARZU[2], E. S. BURSTEIN[3]; [1]Human Sciences,, Univ. Houston-Clear Lake Mail Code 265, Houston, TX; [2]Univ. of Houston-Clear Lake, Houston, TX; [3]ACADIA Pharmaceuticals Inc., San Diego, CA

Abstract: The nicotinic receptor antagonist mecamylamine, 1 mg/kg, precipitates a vigorous withdrawal syndrome only in nicotine-dependent rats. Previous evidence suggests that this syndrome is mediated in part by adaptations of endogenous opiate mechanisms to chronic

nicotine exposure, which triggers release of endogenous opiate peptides. Neuropeptide FF (NPFF) and related neuropeptides contribute to opiate tolerance and dependence through anti-opiate actions, which appears to be primarily mediated by the FF1 receptor. Stimulation of the FF2 receptor primarily induces pro-opiate effects. AC-262620 is a systemically active, selective FF1 receptor antagonist of small molecular weight. Thirteen rats were infused for seven days with 9 mg/kg/day nicotine bitartrate via osmotic minipump, while six were infused with saline alone. Six of the nicotine-dependent rats were injected i.p. with 10 mg/kg AC-262620, while seven received only the saline/DMSO injection vehicle. The non-dependent rats were injected i.p. with saline only. One hour after i.p. injections, all rats were challenged with 1 mg/kg mecamylamine s.c. All subjects were then observed over 30 minutes under blind conditions on a standard checklist of somatically expressed nicotine withdrawal signs. The non-dependent/saline injected group averaged 19.17 ± 2.97 signs (M ± SEM), while the nicotine-dependent rats injected with saline averaged 50.00 ± 7.12 signs. In contrast, the nicotine dependent rats pretreated with AC-262620 averaged only 24.00 ± 4.84 signs. One-way ANOVA revealed a significant group effect, $p = .002$. The AC-262620-pretreated nicotine dependent group had significantly fewer mecamylamine precipitated signs than the nicotine dependent saline-pretreated group, $p = .011$ (Tukey's HSD post-hoc test), but was not significantly different from the non-dependent group, $p = .821$. The results implicate activation of the FF1 NPFF receptor in nicotine dependence and subsequent withdrawal syndrome.

Disclosures: **D.H. Malin:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Acadia Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Acadia Pharmaceuticals. **M.M. Henceroth-Chomiak:** None. **J.J. Izygon:** None. **D.J. McGhiey:** None. **K.M. Bright:** None. **D.M. Nghiem:** None. **P. Goyarzu:** None. **E.S. Burstein:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.04/I29

Topic: C.17. Drugs of Abuse and Addiction

Support: DA 015663

CA 089392

Title: The effect of nicotine administration and withdrawal on sleep in mice

Authors: *H. L. MATHEWS[1], V. GRIMSHAW[2], J. A. STITZEL[3];
[1]Psychology & Neuroscience, IBG, [2]IBG, [3]Integrative Physiology, IBG, Univ. of Colorado - Boulder, Boulder, CO

Abstract: Sleep disturbances are a commonly reported symptom during tobacco cessation attempts. They are currently the only symptom of human nicotine withdrawal syndrome that has not been correlated in a rodent model. The current study investigates the effect of nicotine administration and withdrawal on sleep quantity and quality in a forced oral nicotine mouse model. Nine subjects were implanted with EEG and EMG recording devices using standard procedures. After a recovery and acclimation period, data was recorded continuously for a 4-week period, certain days were chosen over each condition for sleep scoring. Mice had ad libitum access to food and a drinking water solution containing .2% saccharin. Baseline sleep and wake data was scored for three consecutive 24 periods, and subsequently averaged. Immediately following baseline, five of the subjects began receiving 200µg/ml of nicotine for a period of 2 weeks (nicotine group). The control group did not experience any changes. Data for this condition was scored on days 1, 4, 8, 11, and 13. Withdrawal was precipitated spontaneously by excluding the nicotine from the drinking solution; the first two days of withdrawal were scored. Nicotine consumption tended to decrease total sleep. The effect was primarily seem during the lights off period and can mostly be explained by a decrease in time spent in NREM. Additionally, nicotine withdrawal appears to have an effect on the number of stage changes, both the number of awakenings from sleep and the number of total stage changes. The current data suggests of effect of nicotine consumption and withdrawal on the sleep wake cycle.

Disclosures: H.L. Mathews: None. **V. Grimshaw:** None. **J.A. Stitzel:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Pfizer, Inc..

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.05/I30

Topic: C.17. Drugs of Abuse and Addiction

Support: AA018747

IAADR

Title: Glutamate mediates p38-induced presenilin 1 activation

Authors: *M. E. JUNG, D. METZGER;
Univ. N Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: The mutation of presenilin 1 (PS1) genes is known to mediate age-associated brain disorders such as Alzheimer disease. However, little information is available about the interaction between PS1 and stress-activated kinase p38 in response to an excitatory stress. Our previous studies have demonstrated that repeated-withdrawal from a high dose of ethanol consumption provokes the overexpression of p38α (p38 isoform) in the rat brains. Here, we tested the hypothesis that p38α induces the activation of PS1 through glutamate in response to ethanol withdrawal insults. Young adult male rats received an ethanol program, consisting of 4-week-ethanol diet and 3-week-withdrawal per cycle for two cycles. At the end of the diet program, the protein and/or mRNA levels of p38α and PS1 were measured in the prefrontal cortex. Separately, HT22 (mouse hippocampal) cells were exposed to an ethanol program, consisting of 24-hour-ethanol and 4-hour-withdrawal per cycle for two cycles to measure p38α and PS1. Finally, HT22 cells were exposed to glutamate with or without a p38α inhibitor to measure PS1 level. Both ethanol withdrawn rats and HT22 cells show an increase in the protein and mRNA level of p38 and PS1. HT22 cells treated with glutamate also show an increase in the protein level of PS1 in a manner that is attenuated by a p38 inhibitor. These results suggest that p38α activates PS1 through glutamate upon EW. They also suggest that p38α-PS1 link is readily formed under a hyperexcitatory stress.

Disclosures: M.E. Jung: None. **D. Metzger:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.06/I31

Topic: C.17. Drugs of Abuse and Addiction

Support: Supported by a 2014-15 Saint Vincent College Faculty Development Grant to MER.

Title: Tissue hypothalamic-pituitary-adrenal axis responses to nicotine and mecamylamine following *in vivo* continuous nicotine administration and withdrawal

Authors: *M. E. RHODES[1], L. E. HARBAUGH[1], J. A. RUTKAUSKAS[1], R. T. RUBIN[2]; [1]St. Vincent Col., Latrobe, PA; [2]Psychiatry, VA Greater Los Angeles Healthcare Syst., Los Angeles, CA

Abstract: The hypothalamic-pituitary-adrenal (HPA) axis is a three-component endocrine system that modulates physiological responses to stress. To better understand the biology of nicotine (NIC) addiction, we developed an *in vivo* model of continuous NIC administration and withdrawal in laboratory rats, in order to study HPA axis stress responses to NIC. Our earlier studies demonstrated that HPA responses to NIC were reduced and transient following continuous NIC administration, but were enhanced and sustained following NIC "withdrawal"

by mecamylamine (a NIC receptor antagonist). In the present study, following *in vivo* continuous NIC administration and withdrawal, we determined HPA axis hormone responses to NIC and mecamylamine in a three-flask, *in vitro* model of the HPA axis. Hypothalami, pituitaries, and adrenal glands were collected from male rats under two dosing conditions: 1) immediately following 2-week continuous NIC via thrice daily injections plus voluntary consumption of NIC in drinking water (to model nicotine habituation), and 2) 24 h after cessation of 2-week continuous NIC (to model NIC withdrawal). For each axis studied, one-half hypothalamus, one-half pituitary, and one adrenal gland were placed individually into three temperature-controlled flasks connected by tubing and perfused in series with modified Bradbury buffer. Sampling ports between flasks were used to collect buffer at intervals before and after addition of NIC and mecamylamine, for measurement of corticotropin-releasing hormone from the hypothalamus flask, adrenocorticotropic hormone from the pituitary flask, and corticosterone from the adrenal flask. Hormones were measured by highly specific immunoassays. The *in vitro* system maintained stable temperatures, flow rates, pH and hormone baselines. *In vitro* HPA responses were significantly higher in the continuous NIC group than in the NIC withdrawal group. Mecamylamine addition to the hypothalamus flask decreased HPA axis activity in the continuous NIC group but had little effect in the NIC withdrawal group. These results suggest that *in vitro* HPA responses are enhanced and sustained following continuous NIC, and reduced following NIC withdrawal. The findings stand in contrast to our previous *in vivo* results with continuous NIC and its withdrawal, as described above. Further *in vitro* as well as *in vivo* studies addressing the complex relationships among NIC, stress, and the HPA axis may help elucidate new approaches to the understanding and treatment of nicotine addiction.

Disclosures: **M.E. Rhodes:** None. **L.E. Harbaugh:** None. **J.A. Rutkauskas:** None. **R.T. Rubin:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.07/I32

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA023597

NIH grant 036691

The Pearson Center for Alcoholism and Addiction Research

NIH grant DA004398

Title: Activation of the dynorphin-kappa system in the central nucleus of the amygdala mediates the negative emotional state of nicotine withdrawal but not escalation of nicotine intake

Authors: *M. KALLUPI[1], A. COHEN[1], T. E. GRIEDER[2], G. DE GUGLIELMO[1], O. GEORGE[1];
[1]Committee on the Neurobio. of Addictive Disorders The Scripps Res. Instit, Scripps Res. Inst., La Jolla, CA; [2]Inst. of Med. Sci. and Dept. of Mol. Genet., Toronto, ON, Canada

Abstract: Abstinence from nicotine often results in emergence of a negative emotional state that predict relapse and escalation of nicotine intake in humans and rats. Prodynorphin and activation of kappa receptors have been shown to produce withdrawal-like symptoms, suggesting that activation of the dynorphin-kappa system may be responsible for the emergence of a negative emotional state leading to escalation of nicotine intake. However, the causal role of activation of the dynorphin-kappa system on measures of negative emotional states and nicotine intake in dependent animals remains to be demonstrated. To test this hypothesis we tested the effect of systemic blockade of kappa receptors using a long-lasting kappa antagonist (nor-BNI), and downregulation of prodynorphin in the central nucleus of the amygdala using a viral vector (AAV-shPdyn) on withdrawal induced-pain, -conditioned place aversion, escalation of nicotine intake and stress-induced reinstatement. We found that withdrawal-induced hyperalgesia, conditioned place aversion to withdrawal and nicotine escalation, were prevented by nor-BNI (30 mg/kg). Immunohistochemical analysis showed that prodynorphin's content was increased in the CeA in nicotine dependent rats, but not in non-dependent rats. Downregulation of prodynorphin in the CeA using AAV-shPdyn did not affect nicotine escalation, but significantly decreased withdrawal-induced hyperalgesia, aversion to withdrawal and stress-induced reinstatement using the pharmacological stressor Yohimbine (1.25mg/kg). These results demonstrate that while activation of kappa receptors mediates both the negative emotional state of withdrawal and the increased motivation for nicotine after abstinence, increased prodynorphin levels in the CeA only mediates the negative emotional state of nicotine withdrawal, but does not affect the motivation for nicotine. This report provides preclinical evidence for the efficacy of kappa antagonists in reducing the motivational effects of nicotine withdrawal, and identify that upregulation of prodynorphin in the CeA is responsible for the emergence of the negative emotional state of nicotine withdrawal.

Disclosures: M. Kallupi: None. **A. Cohen:** None. **T.E. Grieder:** None. **G. de Guglielmo:** None. **O. George:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.08/I33

Topic: C.17. Drugs of Abuse and Addiction

Support: AA021657

AA022292

Title: Chronic ethanol enhances corticotrophin-releasing factor-dependent potentiation of glutamatergic transmission in lateral habenula neurons

Authors: *J. YE[1], W. ZUO[2];
[2]Anesthesiol., [1]Rutgers, New Jersey Med. Sch., Newark, NJ

Abstract: Background: The lateral habenula (LHb) in the epithalamus acts as an interface between stress- and addiction-related processes. Corticotrophin-releasing factor (CRF) is associated with stress-induced alcohol use disorders. This study was to determine: (1) whether CRF regulates excitatory drive onto LHb neurons; and (2) whether the effect of CRF is altered by chronic ethanol exposure. Methods: We recorded miniature EPSCs (mEPSCs) from the LHb neurons in brain slices from rats at 24-hour withdrawal from either chronic ethanol (CET, 2g/kg, i.p., twice/day, 2 weeks) or saline (SHAM). Results: Acute CRF or ethanol enhanced mEPSCs in LHb neurons in slices from both CET and SHAM groups. CRF (40 nM), which preferentially binds to CRF1 receptors (CRF1Rs), induced a greater mEPSC enhancement in the CET than SHAM group. This is mimicked by stressin 1 (a CRF1R-selective agonist, 1.5nM). Conversely, ethanol-induced a smaller mEPSC enhancement in CET than SHAM group. Interestingly, the CRF1R-selective antagonist (NBI 27914, 1 μM) attenuated the ethanol's enhancement of mEPSCs in both groups, whereas the CRF2-selective antagonist (astressin 2b, 20 nM) and urocortin2 (a CRF2-selective agonist, 1.5 nM) respectively potentiated or suppressed mEPSCs only in the CET group. These results suggest that CRF1R activation in the LHb contributes to CRF- or ethanol-induced mEPSC facilitation. CRF2Rs act opposite to the CRF1Rs after chronic ethanol exposure. This may contribute to the weaker effect of acute ethanol on mEPSCs on LHb during ethanol withdrawal. Conclusion: The CRF system modulates neuroadaptive changes in the LHb circuit during ethanol withdrawal, and both the CRF1 and CRF2 in this area mediate important mechanisms that contribute to ethanol withdrawal.

Disclosures: J. Ye: None. **W. Zuo:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.09/I34

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020073

Title: CaV1.3 channels in inferior colliculus neurons are critical for alcohol withdrawal seizures

Authors: *P. N'GOUEMO;
Pediatrics, Georgetown University Medical Center, Washington, DC

Abstract: The inferior colliculus (IC) is critical in the initiation of acoustically evoked alcohol withdrawal seizures (AWSs), and L-type of voltage-gated Ca2+ (CaV1) channels are thought to contribute to AWS pathogenesis. We have previously reported that CaV1 channel current density was elevated in IC neurons during alcohol withdrawal. Molecular studies demonstrated that CaV1.2α1 subunit mRNA expression outlasted the finite period of AWS susceptibility, whereas CaV1.3α1 subunit mRNA and protein expression occurred before and paralleled the expression of AWSs. Suppresses AWS. Furthermore, pharmacological blockade of CaV1 channels within the IC suppresses AWS. Here I further evaluate the role of CaV1.2 and CaV1.3 channels in rat IC neurons in the pathogenesis of AWSs. Adult, male Sprague-Dawley rats received three daily doses of ethanol (30% v/v in Isomil solution) every eight hours for four consecutive days; control rats received vehicle. Selective siRNA anti-CaV1.2 or siRNA anti-CaV1.3 channels were administered intra-IC daily for four days during ethanol intoxication. Rats were tested for AWS susceptibility 20-24 hours after ethanol withdrawal. AWSs consisted of wild running (WRS) that evolved into generalized bouncing clonic seizures (clonus) and occasionally tonic forelimb extension (TFE). In the control group, the prevalence of WRSs, clonus and TFE was 83%, 58% and 42%, respectively. Quantification shows that intra-IC microinjections of siRNA anti-CaV1.2 reduced the prevalence of WRS, clonus and TFE to 40%, 30% and 20%, respectively. On another token, intra-IC microinjections of siRNA anti-CaV1.3 reduced the prevalence of WRS, clonus and TFE to 13%, 0%, and 0%, respectively. Thus, selective deletion of CaV1.3 but not CaV1.2 channels in IC neurons completely suppresses the occurrence of clonus and tonic seizures. These findings suggest that CaV1.3 L-type channels in IC neurons may play an important role in the pathogenesis of AWSs.

Disclosures: P. N'Gouemo: None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.10/I35

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA15356

NIH Grant AA14445

NIH Grant AA19462

Title: Reduced dendritic complexity in the agranular insular cortex of rats following ethanol exposure and withdrawal

Authors: *M. E. FROST[1], C. W. BIRD[1], V. L. PETERSON[1], B. MCCOOL[2], D. A. HAMILTON[1];
[1]Univ. of New Mexico, Albuquerque, NM; [2]Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: The agranular insular cortex (AID) plays a critical role in social behavior and behavioral flexibility. These behaviors require a distributed set of neural circuitry that includes the amygdala, hippocampus, AID, and other frontocortical regions. Previous work from our laboratory has observed effects of ethanol exposure and withdrawal on dendritic morphology within the nucleus accumbens (NAc) of the adult rat brain (Peterson et al., 2015). Chronic exposure to ethanol followed by a short-term withdrawal period led to decreased measures of neuronal morphological complexity in the NAc core and shell, while a long-term withdrawal period resulted in increased dendritic complexity in the NAc core. The NAc has reciprocal connections to AID, therefore it is reasonable to predict that similar changes in neuronal morphology may be observed within AID. The current study sought to examine the effects of short (24 hour) and long (7 day) withdrawal from chronic ethanol exposure on dendritic morphology in AID. Adult male Sprague Dawley rats were placed in airtight Plexiglas chambers in which they were exposed to ethanol vapor (~37mg/L air) during the light cycle (12hr/day) for 10 consecutive days. This type of exposure produces blood-ethanol concentrations in rats in the 150-200mg/dL range. Control animals only received room air but were otherwise housed in identical conditions for the 10 day exposure period. The ethanol exposed animals were euthanized either 24 hours or 7 days after the 10 consecutive days of exposure. Golgi stained layer II/III pyramidal neurons from AID were traced at 200x magnification. Branch and Sholl analyses were used to analyze dendritic morphology of both apical and basilar dendritic segments. There was a reduction in the complexity of apical branching in the 7 day withdrawal group, but no effects were detected in the basilar field or in length of apical dendrites. There were no significant reductions in dendritic morphology of the 24 hour withdrawal group, however, the numerical reductions in apical length and branching in the 24 hour withdrawal group were intermediate to the 7 day withdrawal group. These data suggest a relationship between withdrawal from ethanol and morphological alterations in dendritic apical length and branching.

Disclosures: **M.E. Frost:** None. **C.W. Bird:** None. **V.L. Peterson:** None. **B. McCool:** None. **D.A. Hamilton:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.11/I36

Topic: C.17. Drugs of Abuse and Addiction

Support: WSU Alcohol Drug Abuse and Research Program Grant

Title: Acute alcohol withdrawal induces sex-dependent alterations in endocannabinoid mRNA expression and negative affect in alcohol dependent rats

Authors: *A. WILLIAMS, A. L. BERGER, R. J. MCLAUGHLIN;
Washington State Univ., Pullman, WA

Abstract: In alcohol dependent individuals, withdrawal is associated with severe affective, physiological, and cognitive symptoms, and research has shown that the endocannabinoid (ECB) system is an important regulator of stress-related affective behaviors observed during acute alcohol withdrawal. Clinical and preclinical evidence demonstrates sex differences in alcohol dependence development, as well as ECB regulation of alcohol dependence, though little research has examined sexually dimorphic changes in ECB related genes during withdrawal. Therefore, this study aimed to assess sex differences in negative affect and changes in ECB related mRNA expression during acute withdrawal in alcohol dependent rats. Specifically, we measured CNR1 and CNR2 mRNA, as well as mRNA for ligands responsible for the synthesis (a specific phospholipase D [NAPE-PLD] and DAG lipase [DGL]) and metabolism (fatty acid amide hydrolase [FAAH] and monoacylglycerol lipase [MGL]) of ECBs in the basolateral amygdala (BLA), medial prefrontal cortex (mPFC), nucleus accumbens (Acb), lateral habenula (LHb), and ventral tegmental area (VTA). Adult male and female rats were exposed to 6 weeks of chronic intermittent alcohol vapor. Following dependence induction, rats were assessed for negative affective behavior in acute withdrawal, and on the subsequent day at the same time point, rats were sacrificed and their brain tissue was harvested for rtqPCR. Compared to control males, alcohol dependent males expressed more air-puff induced 22-kHz ultrasonic vocalizations, an ethologically valid measure of negative affect in rats, and spent less time in the open arm of the elevated plus maze, suggestive of increased anxiety-like behavior. However, these endpoints were not significantly altered in alcohol dependent female rats. Compared to control males, alcohol dependent males also showed decreased CNR1, CNR2, MGL, and DGL mRNA in the BLA, decreased NAPE-PLD and CNR2 mRNA in the mPFC, decreased FAAH and CNR2 mRNA in the LHb, and decreased MGL, NAPE-PLD, DGL, and CNR2 mRNA in the Acb. Notably, these changes were not observed in alcohol dependent female rats. No significant alterations in ECB related genes were observed in the VTA of male or female alcohol-dependent rats. These results demonstrate that withdrawal from chronic intermittent alcohol vapor alters both negative affect and ECB related gene expression in a sexually dimorphic manner. Furthermore, the sex-specific effects seen in alcohol withdrawal-induced negative affect may be the result of sexually dimorphic ECB signaling, which significantly contributes to our understanding of the sex differences observed in alcohol dependence.

Disclosures: **A. Williams:** None. **A.L. Berger:** None. **R.J. McLaughlin:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.12/I37

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R15 AA018213

GVSU Center for Scholarly and Creative Excellence

GVSU Trio McNair Scholars Program

Title: Kappa opioid regulation of depressive-like behavior and ethanol self-administration following protracted withdrawal from ethanol

Authors: D. M. CHAPA, S. K. JARMAN, *G. R. VALDEZ;
Dept Psychol, Grand Valley State Univ., Allendale, MI

Abstract: Withdrawal from alcohol is often characterized by enhanced negative affect, such as symptoms of depression and anxiety. These behavioral changes can be long-lasting in nature, which further contributes to the challenge of the long-term management of relapse prevention. Recent evidence from animal models suggests that the increased activity of the dynorphin (DYN)/kappa opioid receptor (KOR) system leads to an increase in depressive-like behaviors and ethanol self-administration following withdrawal from ethanol. The objective of the present experiments was to determine the role of the KOR system in the regulation of ethanol self-administration and depression-related behaviors following protracted abstinence from ethanol. In the first experiment, male Wistar rats were trained to self-administer ethanol using a saccharin fading procedure. Following this procedure, rats were fed an ethanol or control liquid diet for approximately 4 weeks. Three weeks after removal of the liquid diet, the ability of KOR antagonist nor-BNI decrease ethanol self-administration was examined. At the conclusion of the 3 week withdrawal period, rats were injected with saline, and 24 h later, were allowed to self-administer ethanol. Immediately following this initial self-administration session, animals were pretreated with nor-BNI (20 mg/kg, i.p.), and were again allowed to self-administer ethanol 24 h later. To assess the ability of nor-BNI to attenuate increases in depressive-like behavior, animals were examined in the forced swim test. Male Wistar rats were exposed to an ethanol or control liquid diet as described above. Three weeks after removal of the diet, rats were injected with nor-BNI (20 mg/kg, i.p.), and 24 h later, were exposed to a 10 min session of forced swim stress. The following day, rats were given a 5 min forced swim session that was recorded and examined for time spent immobile. Although rats did not show increased ethanol self-administration during protracted withdrawal from ethanol following saline injections, nor-BNI selectively decreased self-administration in ethanol dependent animals. In the forced swim test, ethanol dependent rats displayed a characteristic increase in time spent immobile compared to control rats, an effect that was reversed by pretreatment with nor-BNI. These results suggest that long-term changes in KOR mechanisms may underlie the depressive-like behavioral changes as well as ethanol self-

administration associated with protracted periods of abstinence from ethanol. **DMC and SKJ contributed equally to this project.

Disclosures: D.M. Chapa: None. **S.K. Jarman:** None. **G.R. Valdez:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.13/I38

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant AA20539

Ralph W. and Grace M. Showalter Research Trust

Title: Protective role of delta opioid receptor in alcohol withdrawal-induced hyperalgesia

Authors: D. ALONGKRONRUSMEE[1], *R. M. VAN RIJN[2,1];
[1]Medicinal Chem. and Mol. Pharmacol., [2]Neurol., Purdue Univ., West Lafayette, IN

Abstract: Chronic pain and hyperalgesia are a major health issue for which adequate treatment options are still lacking. Withdrawal from long-term alcohol consumption can induce hyperalgesia in both humans and rodents. Yet, the molecular mechanisms underlying this phenomenon are not well understood, which stifles development of new therapeutics. We have previously shown that under naïve conditions, delta opioid receptors (DORs) are expressed at a low level in the spinal cord circuits mediating thermal nociception. However, prolonged alcohol exposure can promote a redistribution of DORs to the cell surface and increase potency of DOR selective agonists. We have also shown that DORs are present in mechanical pain-mediating circuits in naïve states. However, alcohol exposure does not increase DOR agonist potency in mechanical antinociception. Interestingly, we have found that alcohol withdrawal-induced mechanical hyperalgesia is exacerbated in DOR knockout mice. This suggests that DORs play a protective role in the establishment and/or expression of alcohol withdrawal induced hyperalgesia and thus could be a promising target for treatment of chronic pain induced by alcohol withdrawal. We hypothesize that DOR expression during withdrawal may be enhanced and that DOR agonists would showed increased potency in reducing mechanical sensitivity during alcohol withdrawal compared to naïve mice. To establish alcohol withdrawal-induced hyperalgesia, mice were exposed to alcohol by oral gavage for three weeks followed by a period of abstinence during which the mice resided in a stable hyperalgesic state lasting more than two weeks. We then intrathecally administered increasing doses of the DOR selective agonists SNC80 and TAN-67 and determined mechanical sensitivity using von Frey filaments. We found that both DOR agonists reduced alcohol withdrawal-induced mechanical hyperalgesia but had reduced potency compared to naïve mice. From our results it is not evident that after withdrawal

or during withdrawal DOR expression and/or function is increased. Therefore we believe that DORs are important during the establishment of alcohol withdrawal-induced hyperalgesia or very early in its expression. Further studies will need to be designed to explore DORs at these time points.

Disclosures: D. Alongkronrusmee: None. **R.M. Van Rijn:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.14/I39

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA018400

NIH Grant AA023305

NSF

LSU Research Enhancement Fund

Title: Inhibitor-2 (I-2) , a regulator of protein phosphatase-1 (PP1), mediates alcohol withdrawal anxiety-like behavior in rats

Authors: *H. YANG[1], H. HOU[1], E. R. HELLARD[2], C. ITOGA[2], B. BAYNES[2], Y. TANG[3], N. W. GILPIN[2], H. XIA[1,3];
[1]Neurosci., [2]Physiol., [3]Cell Biol. and Anat., LSU Hlth. Sci. Ctr., New Orleans, LA

Abstract: Alcohol use disorder is a major health concern in the VA population and in the general population, exerting an enormous toll on the society. Anxiogenic effects of withdrawal after chronic alcohol consumption contribute heavily to the negative affect thought to drive subsequent alcohol-seeking behavior. Plasticity proteins such as the transcription factor CREB, activated via phosphorylation at Ser133 (pCREB), regulate anxiety-like behavior during withdrawal in alcohol-dependent rats by regulating gene expression in the central nucleus of the amygdala (CeA). Protein phosphatase-1 (PP1), a major plasticity gene itself, inactivates CREB by dephosphorylating CREB at Ser133 in neurons. PP1 is critical for memory processes, likely due to CREB-mediated gene expression in hippocampus, but the roles of PP1 and PP1 regulation in anxiety-like behavior and excessive alcohol drinking by alcohol-dependent animals have not been explored. Our recent work has discovered that PP1 inhibitor-2 (I-2) is an important endogenous regulator of PP1 in synaptic plasticity (LTD) and memory formation (novel object recognition and contextual fear conditioning). I-2 mRNA expression level is highest in amygdala, which suggests a critical role of I-2 in amygdala-mediated brain function. Indeed, our preliminary data show that I-2 knockdown in the rat CeA blocked the anxiogenic effect of

withdrawal in alcohol-dependent rats, which indicates that I-2 promotes anxiety-like behavior in the CeA. Moreover, we found that pCREB is increased in primary amygdala neurons when I-2 is knocked-down (KD), consistent with the proposed role of CREB in anxiety-like behavior. Finally we found that I-2 expression is reduced by chronic alcohol treatment, but alcohol withdrawal leads to the rebound of I-2 to control level. Scientific Impact: Our data show that I-2 in the CeA promotes anxiety-like behavior during alcohol withdrawal in alcohol-dependent rats, likely via positive regulation of PP1 function and inactivation of CREB-mediated gene expression. These data may provide insights into future therapeutic interventions in alcohol addiction in humans.

Disclosures: **H. Yang:** None. **H. Hou:** None. **E.R. Hellard:** None. **C. Itoga:** None. **B. Baynes:** None. **Y. Tang:** None. **N.W. Gilpin:** None. **H. Xia:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.15/I40

Topic: C.17. Drugs of Abuse and Addiction

Support: CNPq

CAPES

Propesq-UFRGS

Title: N-acetylcysteine prevents alcohol withdrawal-induced anxiety in rats

Authors: ***R. GOMEZ**[1,2,3,4], R. SCHNEIDER JR[2], S. BANDIERA[3], A. W. HANSEN[3], R. PULCINELLI[1], G. CALETTI[4], E. ELISABETSKY[3];
[1]Univ. Federal Do Rio Grande Do Sul - UFRGS, Porto Alegre, Brazil; [2]Programa de Pós-Graduação em Neurociências, UFRGS - Porto Alegre, RS, Brazil; [3]Programa de Pós-Graduação em Farmacologia e Terapêutica, UFRGS - Porto Alegre, RS, Brazil; [4]Univ. Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Programa de Pós-Graduação em Ciências da Saúde, Porto Alegre RS, Brazil

Abstract: Anxiety and craving, during alcohol withdrawal, have been related to increased levels of corticosterone and leptin. We previously reported that N-acetylcisteine (NAC), a drug that modulates glutamatergic system and presents anti-addictive properties, prevents the alcohol withdrawal-induced increase in serum corticosterone and leptin when administrated for four days after alcohol cessation in rats. Given the relevance for its potential clinical application in alcoholics, the aim of this study was to verify the effects of NAC administered before withdrawal on anxiety behaviors, corticosterone and leptin biomarker in model of moderate alcohol intake in

rats. Male Wistar rats were treated with 2 g/kg of alcohol (20% w/v, n = 30) or glucose solution (n = 30), twice daily, by gavage, 5 days/week for 3 weeks. Four days before alcohol cessation, rats were treated (i.p.) with saline (n = 10) or NAC (60 or 90 mg/kg). Twenty-four hours after alcohol cessation and 20 h after the last NAC administration, rats were exposed to the open field test (OF) for 5 min and behaviors were video recorded. Blood samples were collected and centrifuged for serum corticosterone and leptin analysis by ELISA. A two-way ANOVA, followed by Tuckey test was used to determine differences and Pearson´s test, to verify correlations (behaviors × corticosterone or leptin). Significance was set at $P < 0.05$ and ethics committee approved this study (CEUA-UFRGS: # 23069). Our results showed that alcohol withdrawal decreased the time spent in the central area in the OF ($P = 0.002$), as well as serum corticosterone ($P = 0.019$) and leptin levels ($P = 0.038$). NAC prevented alcohol withdrawal-induced anxiety ($P > 0.05$), and increased serum corticosterone and leptin levels ($P > 0.05$). There was a weak negative correlation between the time spent in the central area in the OF and serum leptin levels ($r = - 0.40$, $P = 0.008$). Using a model of chronic moderate alcohol intake, we herein show that NAC prevented anxiety behaviors and enhanced serum biomarkers induced by alcohol-withdrawal in rats. Moreover, adding to results observed in alcoholics, leptin was inversely correlated with anxiety behaviors in rats suggesting that this hormone may be involved with the negative affect of alcohol withdrawal. Translationally relevant studies can assist in the design of the clinical studies needed to confirm the usefulness of NAC in the management of alcohol withdrawal.

Disclosures: **R. Gomez:** None. **R. Schneider Jr:** None. **S. Bandiera:** None. **A.W. Hansen:** None. **R. Pulcinelli:** None. **G. Caletti:** None. **E. Elisabetsky:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.16/I41

Topic: F.01. Human Cognition and Behavior

Support: AA023165

AA010723

AA012388

AA017168

Title: Failure of abstinent alcoholics to engage frontoparietal control systems when viewing alcohol beverage pictures: A task-activated fMRI study

Authors: *E. M. MULLER-OEHRING[1,2], W. CHU[2], E. V. SULLIVAN[1], A. PFEFFERBAUM[2], T. SCHULTE[2,3];
[1]Dept. Psychiatry & Beh. Sci., Stanford Univ. Sch. of Med., Palo Alto, CA; [2]Biosci., SRI Intl., Menlo Park, CA; [3]Pacific Grad. Sch. of Psychology, Palo Alto Univ., Palo Alto, CA

Abstract: Alcoholism is associated with attentional bias toward alcohol-related stimuli that have gained emotional relevance and processing priority and may have the potential to override attention to nonalcohol-related stimuli. To test this possibility, we used a Match-to-Sample task with functional MRI to identify neural correlates of alcohol and emotional stimulus interference during task processing in 26 participants with alcohol use disorder (AUD) (sober for median=86 days), and 26 age-matched controls. The task was to match the color of a patch to the color of a word by pressing a yes-key for color matches and a no-key for non-matches. Interference from alcohol and emotion stimuli was tested interspersing pictures of alcohol beverages and emotional faces (e.g., happy, angry) between color patches and words. Overall color matching performance (response times and error rates) did not differ between groups, but groups differed in their regional activation patterns that were related to the specific task conditions and clinical characteristics. When alcohol and emotion pictures were presented during color matching (relative to gray-patch trials), controls engaged a frontoparietal network including precuneus, inferior parietal, medial and dorsolateral frontal regions, whereas AUD activated midbrain, limbic, occipital, and cerebellar regions. More activity in visual occipital and parahippocampal regions to alcohol picture trials correlated with younger age at alcoholism onset (Rho=-.43), greater lifetime alcohol consumption (Rho=.41), and greater interference (longer RT difference between alcohol and control condition) from alcohol pictures during color matching (Rho=.44). Further, in AUD lower medial frontal and insula activation to happy faces correlated with longer sobriety (Rho=-.41, Rho=-.44), more depressive symptoms (BDI, Rho=-.45), and interference from positive facial expressions during color matching (Rho=-.46); greater midbrain activation to angry faces was related to higher anxiety levels. Finally, group-by-condition interactions revealed that AUD deactivated frontoparietal executive control areas to alcohol pictures more than to negative facial emotion, whereas controls showed greater activity with greater interference from alcohol beverage pictures (precunues Rho=.40; superior frontal gyrus Rho=.52). These findings provide evidence that AUD activate limbic areas serving emotion processing but do not activate attentional control systems that could help to overcome interference from alcohol-related stimuli. Support: AA023165, AA010723, AA012388, AA017168

Disclosures: E.M. Muller-Oehring: None. **W. Chu:** None. **E.V. Sullivan:** None. **A. Pfefferbaum:** None. **T. Schulte:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.17/I42

Topic: F.03. Motivation and Emotion

Support: R01MH094489

Title: Rostromedial tegmental nucleus (RMTg) mediates aversive consequences of spontaneous morphine withdrawal

Authors: *G. I. ELMER, K. E. RIEGGER, C. L. MAYO, H. PALACOROLLA, D. B. BELL, P. D. SHEPARD;
Psychiatry, Maryland Psychiatric Res. Center, Univ. of Maryland, Baltimore, MD

Abstract: Neuronal circuits that have electrophysiological control over midbrain dopamine (DA) neurons help govern motivated behavior along various positive and negative dimensions. The habenula - rostromedial tegmental nucleus - ventral tegmental area circuit (Hb-RMTg-VTA) plays a prominent role in modulating these neurons. Projections from Hb to RMTg excite RMTg neurons. These neurons in turn give rise to dense GABAergic projections to midbrain DA neurons, presumably signaling negative events. RMTg neurons are densely populated with μ-opioid receptors and play a role in opioid regulation of DA neurons via disinhibition. The RMTg has been implicated in mediating the rewarding effects of opioid agonists, however its role in the negative consequences of opioid withdrawal are unknown. In the present study we explored the influence of RMTg neurons on the negative consequences of spontaneous opioid withdrawal on the reward system using brain stimulation reward (BSR). Sham (n=6) and RMTg lesioned (n=8) (quinolinic acid; 0.4M in 166nl saline 0.9%) Sprague-Dawley rats were surgically implanted with monopolar electrodes into the medial forebrain bundle (MFB). Rats were trained to nose-poke for rewarding brain stimulation. Alterations in brain reward sensitivity were assessed using a rate-frequency protocol; the shift in the frequency that maintained half-maximal response rates (HMF) quantified the change in sensitivity. Equivalent rate-frequency curves were established in Sham and RMTg lesioned rats using similar currents (~200 μA). HMF values were assessed immediately following and 24hrs after morphine administration (0, 3.0, 5.6, 10.0 or 30.0 mg/kg s.c.). The reward potentiating effects of the low morphine dose was minimal in Sham and Lesioned rats. The aversive consequences of the higher doses were evident only in the Sham animals as evidenced by a dose-dependent increase HMF. Twenty-four hours following morphine administration (acute spontaneous withdrawal) there was a significant dose-dependent increase in HMF that was again only evident only in the Sham animals. Thus, RMTg lesion eliminated the aversive effects of acute high dose morphine and spontaneous morphine withdrawal. Previous studies have demonstrated a role for the RMTg in mediating behavioral reaction to aversive events. The present study highlights the role played by the RMTg in mediating the aversive consequences of acute high dose morphine and spontaneous morphine withdrawal. The results support the notion that RMTg may play a role in negative emotional states during drug-withdrawal and contribute to compulsive drug-seeking.

Disclosures: G.I. Elmer: None. **K.E. Riegger:** None. **C.L. Mayo:** None. **H. Palacorolla:** None. **D.B. Bell:** None. **P.D. Shepard:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.18/I43

Topic: C.17. Drugs of Abuse and Addiction

Support: Natural Science and Engineering Research Council

Canadian Foundation for Innovation

Canadian Institutes of Health Research

Title: Microglial P2X7 receptor activity is gated by morphine-induced phosphorylation

Authors: *H. L. LEDUC-PESSAH[1], N. L. WEILINGER[2], C. Y. FAN[3], N. E. BURMA[3], R. J. THOMPSON[2], T. TRANG[3];
[2]Cell Biol. and Anat., [3]Physiol. and Pharmacol., [1]Univ. of Calgary, Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Morphine is indispensable in the treatment of acute and chronic pain. However, its use is limited by the development of analgesic tolerance, such that higher and more frequent doses are required to achieve the same level of pain control. Growing evidence suggests that microglia are critically involved in the development of morphine tolerance. In the present study, we examined the expression and function of microglial ATP-gated P2X7 receptors (P2X7Rs) after repeated morphine treatment, and their role in the development of morphine analgesic tolerance. In adult male Sprague Dawley rats, repeated morphine administration caused a progressive decline in morphine anti-nociception and a loss in morphine analgesic potency. The development of analgesic tolerance correlated with proliferation and activation of spinal microglia and an up-regulation of total spinal P2X7R expression. In the spinal dorsal horn we found that P2X7Rs are predominantly expressed on microglia using both immunohistochemical co-localization and flow cytometry. Intrathecal administration of the selective P2X7R antagonist, A740003, significantly attenuated of the development of analgesic tolerance. To further characterize the effects of morphine on microglial P2X7Rs, we used the BV2 microglial cell line and found an up-regulation of total P2X7R expression with repeated morphine administration consistent with our observation in spinal cord lysates. The morphine-induced increase in P2X7R protein expression was concomitant with a potentiation of BzATP evoked P2X7R calcium responses and inward current. Both the increase in P2X7R expression and potentiation of function were mediated by μ opioid receptor activation. We next tested whether receptor phosphorylation played a role in the potentiation of P2X7R function. Daily co-administration of a protein kinase inhibitor with morphine prevented the morphine-induced potentiation of P2X7R BzATP-evoked calcium influx and inward current. Taken together, our findings demonstrate that microglial P2X7Rs are causally involved in the development of morphine analgesic tolerance. In addition, we found that

repeated morphine increases total microglial P2X7R expression and induces a phosphorylation mediated potentiation of P2X7R ion channel function.

Disclosures: H.L. Leduc-Pessah: None. **N.L. Weilinger:** None. **C.Y. Fan:** None. **N.E. Burma:** None. **R.J. Thompson:** None. **T. Trang:** None.

Poster

313. Mechanisms of Withdrawal from Alcohol, Nicotine and Morphine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 313.19/I44

Topic: C.17. Drugs of Abuse and Addiction

Support: Canadian Foundation for Innovation

Canadian Institute of Health Research

Title: Morphine withdrawal critically involves spinal microglial P2X7 receptors

Authors: *N. E. BURMA[1], H. L. LEDUC-PESSAH[2], Z. F. CAIRNCROSS[2], T. TRANG[2]; [2]Physiol. and Pharmacol., [1]Univ. of Calgary, Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Opioids, such as morphine, are among the most effective and widely prescribed analgesics for the management of pain. However, their repeated use can lead to opioid physical dependence, which manifests as a withdrawal syndrome upon discontinuing drug use. Converging evidence suggests that opioid withdrawal is critically mediated by changes in the spinal dorsal horn, which is a primary site of action for opioid analgesia. The present study examines the importance of spinal ATP-gated P2X7 receptors (P2X7Rs) in the expression of morphine withdrawal. We treated rats with escalating doses of morphine for 5 days. On day 5, rats received a single injection of morphine and 2 hours later were challenged with an injection of the opioid receptor antagonist, naloxone, to rapidly precipitate withdrawal. We found that morphine treated animals displayed a robust naloxone precipitated withdrawal syndrome characterized by autonomic and somatic hyperactivity. Moreover, in morphine-withdrawn animals we detected a marked increase in spinal P2X7R protein expression. To assess the role of spinal P2X7Rs in morphine withdrawal, we intrathecally injected the selective P2X7R antagonist, A740003, 1-hour prior to naloxone challenge, and found that this acute injection significantly attenuated the morphine withdrawal syndrome. In morphine withdrawn rats we found an increase in spinal expression of CD11b, a cellular correlate of microglial activation. P2X7Rs are highly expressed on microglia within the spinal cord, so we next asked whether the up-regulation of P2X7Rs following morphine withdrawal was localized to microglia. Using flow cytometry, we found that in the CD11b positive population there was a significant increase in the mean P2X7R fluorescent intensity in morphine treated rats. This increase in P2X7R expression was not observed in the CD11b negative population. To further investigate the mechanism by

which P2X7Rs respond to morphine, we used primary rat microglial cultures and the immortalized BV2 microglial cell line. Following repeated morphine treatment, we found that both P2X7R expression and function were increased in primary and BV2 microglia. Collectively, our findings reveal a critical role for spinal microglial P2X7Rs in the expression of morphine withdrawal.

Disclosures: **N.E. Burma:** None. **H.L. Leduc-Pessah:** None. **Z.F. Cairncross:** None. **T. Trang:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.01/I45

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA013983

NIH Grant AA021622

Title: Escalated alcohol drinking in socially stressed mice: CRF-R1 in the ventral tegmental area

Authors: ***L. S. HWA**, E. N. HOLLY, E. ZHANG, J. F. DEBOLD, K. A. MICZEK; Psychology, Tufts Univ., Medford, MA

Abstract: The stress neuropeptide corticotropin-releasing factor (CRF) and its two receptors type-1 and type-2 (CRF-R1 and CRF-R2) are activated during acute as well as repeated stress and alcohol (EtOH) withdrawal. We aimed to study extrahypothalamic CRF-R1/R2 in the dopaminergic ventral tegmental area (VTA) in EtOH drinking that was escalated by social defeat stress and intermittent access to EtOH. Adult, male C57BL/6J mice were exposed to 30 attack bites for ten consecutive days by an aggressive resident. After ten days rest, this first group of mice (group A) was tested for EtOH drinking using intermittent two-bottle choice to 20% EtOH (w/v) and water. Pretreatment with a CRF-R1 antagonist, 17 mg/kg CP376395 ip, before each confrontation was given to a second cohort of socially defeated mice (group B). Two groups of mice were not defeated but consumed EtOH on an intermittent or continuous schedule (groups C and D). After four weeks of EtOH drinking, group A, C and D mice were implanted with bilateral cannulae into the ventral tegmental area (VTA) for either microinjections of the CRF-R1 antagonist CP376395 (0-0.6 µg) or the CRF-R2 antagonist astressin2B (0-0.5 µg). Separate groups of mice were fitted with a probe in the nucleus accumbens for *in vivo* microdialysis of dopamine. Mice with a history of social defeat stress (groups A and B) increased their daily EtOH drinking (ca. 28 g/kg/day) further than the non-defeated controls. Also, pretreatment with CP376395 before confrontations reduced the increased EtOH drinking in mice with defeat experience (group B). The intermittent EtOH access mice (group C) significantly escalated EtOH

intake compared to those on a continuous schedule (group D). We found that CP376395 intra-VTA dose-dependently reduced intermittent EtOH drinking in the stressed and non-stressed mice, but did not affect continuous EtOH intake. Also, astressin2B microinjection into the VTA non-specifically reduced both EtOH and water drinking in the stressed intermittently drinking mice, but not in the non-stressed groups. Nucleus accumbens tonic dopamine levels were elevated in the mice that were socially defeated and given intermittent EtOH compared to the non-defeated drinking mice. After an intra-VTA 0.6 μg CP376395 microinjection, dopamine significantly increased compared to baseline, again only in the stressed condition. These experiments confirm a critical role for VTA CRF-R1, but not CRF-R2, in mediating stress-induced EtOH consumption. VTA CRF-R1 signaling in mesolimbic structures may be a critical node for both reward- and stress-related pathways.

Disclosures: L.S. Hwa: None. **E.N. Holly:** None. **E. Zhang:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.02/I46

Topic: C.17. Drugs of Abuse and Addiction

Title: Stress modulation of alcohol consumption and anxiety-like behavior in specially bred alcohol-preferring and non-preferring rats

Authors: T. O'CONNOR[1], D. CRETHERS[1], *T. B. PATTON[2];
[1]Psychological Sci., [2]Psychology, Georgia Regents Univ., Augusta, GA

Abstract: Disorders characterized by significant symptoms of anxiety, such as post-traumatic stress disorder (PTSD), are often exacerbated by one or more co-occurring disorders. In particularly, alcohol use disorder (AUD) is reported with PTSD at alarming rates. In addition, the finding that exposure to trauma increases the subsequent risk for developing AUD has been well documented. Such high comorbidity of AUD and PTSD coupled with overlapping irregularities in certain neurotransmitters and brain structures suggests that a common underlying pathology exists between these two disorders. Indeed, studies show that certain brain regions and neurocircuitry, such as the prefrontal cortex, hippocampus, and amygdala appear to be dysregulated for both disorders. Distinct lines of research focusing on PTSD and AUD individually have demonstrated a link between impaired plasticity related immediate-early gene (IEG) expression and more severely affected individuals. These findings raise the possibility that such plasticity-related IEGs may be associated with susceptibility to both PTSD and AUD. However, the exact mechanisms that predispose an individual to develop PTSD and/or AUD remain elusive. Here, we wanted to know if a predisposition to drink alcohol would correlate

with impaired fear extinction. To study this, we exposed rats that have been bred to drink large quantities of alcohol to a stressor (tone paired with mild footshook). Alcohol consumption and freezing behavior was measured before and after the alcohol preferring (P) rats were exposed to the stressor. Comparisons were made to rats that have been specially bred to have a low affinity for alcohol, non-preferring (nP) rats. After behavior testing, all brains were processed for activity patterns of the IEG, Homer 1a one of several IEGs that appears to be involved with formation and maintenance of long term changes in synaptic act. As expected, the amount of alcohol consumed by P and nP rats was significantly different before and after exposure to the stressor. In addition, differences were found in the quantity and duration of anxiety-like behaviors between P and nP rats. Contrary to our hypothesis, nP rats displayed higher levels of freezing to the conditioned stimulus (tone) compared to P rats. Fluorescence *in situ* hybridization of the prefrontal cortex revealed clear differences between these two rat breeds. Analyses of hippocampus and amygdala IEG expression will be presented as well. These findings provide further insight into common neuropathaologies of PTSD and AUD.

Disclosures: **T. O'Connor:** None. **D. Crethers:** None. **T.B. Patton:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.03/I47

Topic: C.17. Drugs of Abuse and Addiction

Title: The effect of stress on the acquisition of ethanol self-administration in rats

Authors: *C. J. HEYSER, B. HOFF;
Neurosciences, UCSD, La Jolla, CA

Abstract: The hypothesis that stressful life experiences may contribute to an individual's vulnerability to drug and alcohol abuse has received considerable attention. However, to date the results in animal models has been mixed, with many studies reporting a reduction in ethanol intake following stress. Clearly this is a complex issue as the impact of stress can depend on the magnitude of stress, its predictability, the control an organism has over it and the social environment (context). Therefore, the present study was conducted to examine the effects of unpredictable stressor exposure on the acquisition of ethanol self-administration in male Wistar rats. All rats were given two-bottle (ethanol and water) access (1 hr/day or 24 hr/day) to gradually increasing sweetened (saccharin) ethanol concentrations over a 12-week period. The final concentration of ethanol was 10% w/v. The rats were either individually housed or group housed in pairs during their access to ethanol. Animals assigned to the stress condition were exposed to unpredictable mild stressors daily (e.g., cage crowding, forced swim, cage tilt, strobic light, etc) throughout the period of the experiment. Overall, the results showed that animals

exposed to stressful conditions drank significantly less ethanol than control animals, however this result was specific to the social environment. More specifically, the stress-induced reduction in ethanol intake was greatest in animals that were group housed during their access to ethanol. In contrast, stress had little to no effect on ethanol consumption in individually housed animals. Our working hypothesis is: 1) group housed rats form a fairly stable social structure (dominance hierarchy), 2) exposure to unpredictable stressors may alter the behavior of the rats, resulting in perturbations to the established dominance hierarchy, 3) instability in the social structure along with continued stressor presentation reduces ethanol intake during acquisition. These results strengthen the hypothesis that stress can modify ethanol consumption and that this influence is dependent on the organism's history of ethanol intake and its social environment.

Disclosures: C.J. Heyser: None. **B. Hoff:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.04/I48

Topic: C.17. Drugs of Abuse and Addiction

Title: Stress promotes alcohol consumption and excitatory GABA transmission in the VTA

Authors: *A. OSTROUMOV, A. THOMAS, W. DOYON, B. KIMMEY, J. DANI;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Although stress has been shown to promote alcohol intake in humans, the synaptic and circuit-level mechanisms contributing to this interaction remain largely unknown. We examined the effect of a single restraint stress on alcohol self-administration and on alcohol-induced dopamine (DA) signaling via *in vivo* microdialysis and *in vitro* electrophysiology. Application of restraint stress 15 hours prior to alcohol exposure significantly increased alcohol self-administration over many days and decreased alcohol-induced DA signaling in the nucleus accumbens. *In vitro* patch electrode recordings showed that this decreased DA signaling was mediated by increased GABAergic inhibition onto DA neurons in the ventral tegmental area (VTA). Following stress, local VTA GABA neurons showed a higher firing rate in response to alcohol compared to non-stressed controls. The increase in alcohol-induced GABA neuron firing after stress was blocked by bath application of picrotoxin, suggesting GABAA receptor-mediated excitation. In support, we found that stress caused a positive shift in the reversal potential of GABAA receptors located on VTA GABA neurons, an effect that could contribute to cell excitability in the presence of alcohol. To test this hypothesis, we blocked GABAA receptor-mediated excitation with acetazolamide. Acetazolamide prevented the effect of stress on alcohol-induced neurotransmission *in vitro* and *in vivo*. These findings provide evidence that acute stress switches GABAA receptor function from inhibitory to excitatory in the presence of alcohol,

which significantly alters DA neuron signaling and may contribute to increased alcohol self-administration.

Disclosures: **A. Ostroumov:** None. **A. Thomas:** None. **W. Doyon:** None. **B. Kimmey:** None. **J. Dani:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.05/J1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA014095

NIH Grant AA020929

NIH Grant AA010761

NIH Grant AA007474

VA Medical Research

Title: Combined chronic intermittent ethanol exposure and stress exposure alter micro-RNA expression in prefrontal cortex and hippocampus of C57BL/6J Mice

Authors: M. G. SOLOMON[1], M. P. OVERSTREET[1], R. MCCANN[1], R. I. ANDERSON[1], *M. F. LOPEZ[2], H. C. BECKER[1];
[1]Charleston Alcohol Res. Ctr, Psychiatry and Behavioral Sci., [2]Psychiatry, Med. Univ. of South Carolina, Charleston, SC

Abstract: The neurotrophic factor BDNF has been implicated in pharmacological and motivational effects of ethanol. Expression of BDNF is known to be modulated by multiple microRNAs (miRNAs), including miR-206 and miR-30a-5p. We have previously shown that a history of repeated cycles of chronic intermittent ethanol (CIE) vapor exposure significantly reduced Bdnf and elevated mir-206 mRNA levels in medial prefrontal cortex (mPFC) and hippocampus (HPC), with expression returning to control levels one week after the final CIE exposure. In separate animals, a similar profile was observed after acute forced swim stress (FSS) exposure; i.e. reduced Bdnf and increased miR-206 mRNA levels in both mPFC and HPC. The present study extends these findings by examining effects of combined CIE exposure and acute or repeated FSS exposure on miRNAs that modulate Bdnf mRNA expression. Adult male C57BL/6J mice received 4 consecutive weeks of CIE vapor exposure (EtOH group) or air exposure (CTL group) in inhalation chambers (16 hr/day x 4 days/week). Three days following the final (4th) CIE cycle, half of the subjects were subjected to FSS, with one group sacrificed

after a single 10-min FSS exposure and another group sacrificed after 5 daily FSS exposures. Stressed mice were sacrificed 30 min after the final FSS. The remaining non-stressed subjects were sacrificed at corresponding time points. mPFC and HPC samples were rapidly dissected on ice and miR-206 and miR-30a-5p expression determined by real time quantitative PCR (qRT-PCR), with U6 used as the miRNA reference RNA. In the mPFC, the combination of CIE exposure with either acute or repeated FSS exposure had no effect on miR-206 expression, whereas the combination of CIE exposure and repeated (but not acute) FSS exposure increased miR-30a-5p levels (63±26% increase). In the HPC, the combination of CIE exposure and FSS increased mir-206 expression, with the degree of increase similar for acute FSS (55±30% increase) and repeated FSS (55±24% increase). These results suggest that combined chronic ethanol (CIE) exposure and stress (FSS) challenge produce dynamic alterations in expression of miRNAs that target Bdnf expression, with effects dependent on brain region and acute vs. chronic stress experience. Samples are currently being analyzed to examine effects of combined CIE exposure with acute and repeated FSS exposure on miR-30a-5p expression in HPC. Studies also are planned to directly compare effects of CIE exposure alone, FSS exposure alone, and the combination of CIE and FSS exposure on Bdnf and miRNA expression in these brain regions.

Disclosures: **M.G. Solomon:** None. **M.P. Overstreet:** None. **R. McCann:** None. **R.I. anderson:** None. **M.F. Lopez:** None. **H.C. Becker:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.06/J2

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA HS8339

NIMH NS073574

P30 NS047243

Title: Optical stimulation of CRF-positive neurons in the pVTA decreases later ethanol drinking in mice

Authors: ***L. M. DARNIEDER**[1], K. GOBROGGE[2], L. C. MELON[3], J. MAGUIRE[3], K. A. MICZEK[2];
[1]Tufts Univ. Sackler Sch. of Grad. Biomed, Boston, MA; [2]Psychology, Tufts Univ., Medford, MA; [3]Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Stress can escalate alcohol drinking in susceptible individuals and has been modeled preclinically by exposing mice to social stress. Underlying these effects are the actions of the

stress-induced neuropeptide, corticotropin-releasing factor (CRF), which both governs the HPA axis and has actions at extrahypothalamic sites. Critically, we have shown the importance of CRF signaling within the ventral tegmental area (VTA) to persistent, voluntary escalation of ethanol consumption. As the VTA is crucial to reward circuitry, CRF signaling in the VTA may be a critical region where stress and reward are integrated. However, the exact relationship between local CRF release within the VTA and later alcohol consumption is still unclear. To clarify the role of CRF within the VTA and its effects on later alcohol drinking, we employed an optogenetic strategy to specifically activate CRF neurons in the VTA. Two transgenic lines_floxed-Channelrhodopsin2 (ChR2) and CRF-Cre_were crossed to generate progeny expressing ChR2 specifically in CRF neurons. Male and female offspring were stereotaxically implanted at eight- to ten-weeks of age with optical fibers directed towards the ventral posterior medial VTA (pVTA). Both our lab and others have identified this subregion of the VTA as containing CRF and CRF receptor subtypes. After one week of recovery, ChR2-positive and Cre-negative littermate controls were given either 10 or 30 min of continuous, daily stimulation (5ms, 20 Hz, 473nm laser pulses) intermittently for 10 consecutive days. These parameters were chosen to simulate our social defeat protocol, which has been previously shown to escalate voluntary ethanol consumption. After 10 additional days of no stimulation, mice were subjected to a two-bottle choice, continuous access alcohol (20%, v/v) drinking protocol for six weeks. Results indicate that mice receiving more frequent optical stimulation of the pVTA had reduced ethanol drinking (4.5 g/kg/24h) when compared to optically stimulated, Cre-negative controls (15.49 g/kg/24h). Based on our results, we hypothesize that optically stimulating CRF-positive neurons within the VTA modulates mesolimbic dopamine pathways and mimics the effects of episodic social stress to affect later alcohol consumption.

Disclosures: L.M. Darnieder: None. **K. Gobrogge:** None. **L.C. Melon:** None. **J. Maguire:** None. **K.A. Miczek:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.07/J3

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R37 AA08757

Title: Alcohol programs the stress axis to hyper-response to a future stress challenge from prenatal to pre-pubertal period and not after puberty

Authors: S. MURUGAN[1], M. CABRERA[1], *L. G. CHASTAIN[2], D. K. SARKAR[1]; [1]Animal Sci., Rutgers, the State Univ. of New Jersey, New Brunswick, NJ; [2]The Endocrine Program, Dept. of Animal Sci., Rutgers, The State Univ. of New Jersey, New Brunswick, NJ

Abstract: Alcohol exposure (either prenatally or in the early postnatal period) can impact developmental pathways resulting in lasting structural and regulatory changes that predispose individuals to adulthood disease including long-term hyperresponsiveness to stress with exaggerated circulating glucocorticoids, enhanced anxiety, and depression-like behaviors. Recently, it has been shown that alcohol exposures during the adolescent period similarly predisposes individuals to adulthood stress abnormalities including long-term hyperresponsiveness to stress with exaggerated circulating glucocorticoids and enhanced anxiety. This raises the question of developmental timing, when does alcohol programming of the stress axis to hyper-response cease? To address this issue we fed young rats at various stages of reproductive development (postnatal period 2-7 days of age; juvenile periods, 15-20 days of age; prepubertal period; 23- 28 days of age; or pubertal period 50-54 days age) with a liquid diet containing 11.34% alcohol to raise blood alcohol levels at the range of 150-200 mg/dl. Control animals were pair-fed an isocaloric volume of maltose dextrin. These rats were maintained in the animal house and challenged with restraint stress around 70 days of age. The stress challenge was conducted in both sexes. For females, the stress study was conducted on diestrus. Determination of plasma glucocorticoid levels at various time points after restraint demonstrated an enhanced response to restraint stress in both male and female alcohol-fed animals until pre-pubertal period and not after puberty. These results identify a critical period for alcohol developmental programming of the stress axis.

Disclosures: **S. Murugan:** None. **M. Cabrera:** None. **L.G. Chastain:** None. **D.K. Sarkar:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.08/J4

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant U01 AA020929

NIH Grant U01 AA014095

NIH Grant P50 AA010761

VAMC

Title: CRF1R antagonism reduces ethanol consumption in models of binge drinking, relapse drinking, and stress-induced drinking in adult male C57BL/6J mice

Authors: *R. I. ANDERSON[1], M. F. LOPEZ[2], H. C. BECKER[2];
[1]Ctr. for Drug and Alcohol Programs, [2]Charleston Alcohol Res. Ctr., Med. Univ. of South Carolina, Charleston, SC

Abstract: Stress-related neuropeptides, such as corticotropin releasing factor (CRF), have been strongly implicated in excessive alcohol consumption and the development of alcohol dependence. The present series of experiments was designed to characterize effects of CRF1R antagonism on binge-like drinking, relapse drinking, and stress-induced drinking in adult male C57BL/6J mice. Mice in Experiment 1 were subjected to multiple cycles of a drinking-in-the-dark (DID) procedure (access to a single bottle containing 20% ethanol for 2 hr on days 1-3, and 4 hr on day 4). During the final week of DID, mice were injected with MTIP (0, 20, 40 mg/kg, i.p.) 30 min prior to the day 4 drinking session. The same mice were also later tested for locomotor activity 30 min after the same doses of MTIP. The 40 mg/kg dose of MTIP produced a significant decrease in binge-like ethanol consumption and reduced resultant BEC by 50% without influencing locomotor activity. Mice in Experiment 2 were tested in a chronic intermittent ethanol (CIE) drinking paradigm, a model of ethanol dependence and relapse drinking. Once stable baseline ethanol intake was established in a 2-bottle (15% ethanol vs. water) limited access (2 hr/day) procedure, mice received chronic intermittent exposure (16 hr/day x 4 days/week) to ethanol vapor (CIE group) or air (CTL group). The weekly cycles of inhalation exposure were alternated with 5-day drinking test cycles. No drug treatment occurred during Tests 1-3. As expected, mice in the CIE group consumed significantly higher levels of ethanol than CTL mice. During Test 4, MTIP (0, 5, 10, 20, 40 mg/kg, i.p.) was administered 30 min prior to each daily drinking session. At doses of 10 mg/kg and higher, MTIP reduced ethanol consumption in CIE-exposed mice while not influencing consumption in CTL mice. Experiment 3 examined the effects of MTIP on stress enhancement of CIE-induced escalated drinking. Mice in the stressed condition were subjected to a 10-min forced swim 4 hr prior to each 2-hr drinking session. During Test 5, mice were administered a 40 mg/kg dose of MTIP 10 min prior to the forced swim. MTIP prevented stress-induced elevation of drinking in CIE mice (but not CTL mice). Overall, these results support a role for CRF antagonism not only in models of excessive ethanol consumption, but also in stress enhancement of drinking in the context of dependence. The CRF system appears to remain an appealing therapeutic target for potential treatment of alcohol use disorders.

Disclosures: R.I. Anderson: None. **M.F. Lopez:** None. **H.C. Becker:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.09/J5

Topic: C.17. Drugs of Abuse and Addiction

Support: IN224314 from DGAPA-UNAM to OPG

Title: Alcohol induces changes in the endocannabinoid and dopaminergic systems and concentration of GABA and glutamate in the nucleus accumbens of rats with maternal separation and early stress

Authors: *A. ROMANO[1], L. ALVARADO-CAPULEÑO[2], M. MÉNDEZ-DÍAZ[3], A. RUIZ-CONTRERAS[4], O. PROSPÉRO-GARCÍA[5];
[1]Univ. Nacional Autonoma De Mexico, Mexico, Mexico; [2]Univ. Nacional Autonoma De Mexico Grupo de Neurociencias, Lab. de Canabinoides, Mexico, Mexico; [3]Univ. Nacional Autonoma De Mexico Grupo de Neurociencias UNAM. Lab. De Canabinoides, Mexico, Mexico; [4]Psychology, Univ. Nacional Autonoma De Mexico, Lab.Neurogenomica Cognitiva, Mexico, Mexico; [5]Univ. Nacional Autonoma De Mexico, Grupo de Neurociencias Lab. de Canabinoides, Mexico, Mexico

Abstract: Maternal separation and its associated early life stress (MSS) affect the endocannabinoid system thereby facilitating alcohol consumption. The consequences of alcohol consumption in the brain cannabinoid and other systems of MSS rats has not been fully studied, therefore we decided to describe such potential changes. Results showed that maternal separation (MSS+W) increased CB1R compared to NMS+W. Alcohol increased CB1R in both NMS+EtOH and MSS+EtOH rats in the nucleus accumbens compared to NMS+W rats. Both maternal separation (MSS+W) ands alcohol (NMS+EtOH) induced a reduction in the expression of FAAH. In contrast, alcohol induced in MSS+EtOH rats a similar expression of FAAH than that observed in NMS+W. Maternal separation (MSS+W) induced an increased of GABA; while alcohol reduced GABA in both NMS+EtOH and MSS+EtOH. The MS+EtOH rats exhibited similar levels of GABA than those observed in NMS+W. Alcohol increased glutamate in both NMS+EtOH and MSS+EtOH in the nucleus accumbens compared with NMS+W. MSS+EtOH rats showed an increase in the expression of MeCP2 protein compared with NMS+W. Likewise, alcohol increased D2 receptor in NMS+EtOH while reducing it in MSS+EtOH. Maternal separation (MSS+W) reduced the expression of D3 receptors but alcohol (MSS+EtOH) reversed it to a level similar to NMS+W.All these maternal separation-induced changes seem to promote alcohol consumption, potentially seeking to reverse them.

Disclosures: A. Romano: None. **L. Alvarado-Capuleño:** None. **M. Méndez-Díaz:** None. **A. Ruiz-Contreras:** None. **O. Prospéro-García:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.10/J6

Topic: C.17. Drugs of Abuse and Addiction

Support: National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2014R1A2A2A01005851)

Title: Acupuncture attenuates increases in anxiety-like behaviors and ethanol self-administration in dependent rats via activation of the endogenous opioid system

Authors: *C. YANG, S. CHANG, J. KIM, Y. GWAK, J. LEE, J. LEE, B. LEE, H. KIM; Daegu Haany Univ., Suseong-Gu, Daegu, Korea, Republic of

Abstract: Withdrawal from chronic ethanol reduces mesolimbic dopamine neurotransmission that may represent the mechanism, at least in part, underlying anxiety and depression that accompanies ethanol withdrawal and might also contribute to the intense ethanol craving experienced by addicts. Our previous studies have shown that acupuncture at Shenmen (HT7) points suppresses the reduction of extracellular dopamine levels in the nucleus accumbens (NAc) and withdrawal signs during ethanol withdrawal. The aim of this study was to evaluate the effects of HT7 acupuncture on anxiety-like behaviors and ethanol self-administration during acute ethanol withdrawal in dependent rats. A role for the endogenous opioid system in ethanol and acupuncture effects is also explored. Male Wistar rats were made dependent on ethanol via chronic exposure to ethanol containing liquid diet. Behaviors in the elevated plus maze and ethanol self-administration were measured after acupuncture at bilateral HT7 points followed by 2 h of ethanol withdrawal. The role of β-endorphin in acupuncture effects on these behaviors was examined by local injection of β-endorphin into the nucleus accumbens. Results showed that acupuncture attenuated increases in open-arm exploration behavior and ethanol self-administration during withdrawal in ethanol-dependent rats. Local injection of β-endorphin into the NAc enhanced open-arm exploration and ethanol self-administration behaviors in ethanol-withdrawn rats. HT7 stimulation reduced ethanol suppression of neuronal activity in the arcuate nucleus of the hypothalamus and the decrease in β-endorphin release in the nucleus accumbens in ethanol-withdrawn rats. Furthermore, HT7 acupuncture increased c-fos expression in neurons in the arcuate nucleus projecting to the NAc. Given opioid-dopaminergic interactions in the NAc, these findings suggest that acupuncture may attenuate anxiety-like behaviors and ethanol-seeking behaviors through activation of β-endorphinergic input to the nucleus accumbens from the hypothalamic arcuate nucleus.

Disclosures: C. Yang: None. **S. Chang:** None. **J. Kim:** None. **Y. Gwak:** None. **J. Lee:** None. **J. Lee:** None. **B. Lee:** None. **H. Kim:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.11/J7

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA021802

NIH Grant AA06420

Title: Sex differences in alcohol and corticosterone modulation of glutamatergic input from the basolateral to the central nucleus of the amygdala

Authors: *M. L. LOGRIP, C. OLEATA, M. ROBERTO;
Committee on the Neurobio. of Addictive Disorders, The Scripps Res. Inst., La Jolla, CA

Abstract: Alcohol use disorders are pervasive psychiatric conditions characterized by high rates of relapse. The propensity to relapse can be exacerbated by stress, and females show a greater association between past stress and likelihood of relapse (Hefner et al. 2011 Am J Addict 20:307). Greater sensitivity of females to stress effects on alcohol drinking suggests sexual dimorphism in stress-sensitive brain regions, like the amygdala. We previously presented data demonstrating sex differences in the ability of acute 44 mM alcohol treatment to reduce the amplitude of basolateral amygdala (BLA) -evoked glutamatergic potentials in the lateral (CeL) and medial (CeM) central amygdala (CeA). We showed that alcohol reduced the amplitude of BLA-evoked excitatory postsynaptic potentials (eEPSPs) in the CeL of males, but this effect was reduced in females. In addition, alcohol's reduction of eEPSP amplitude was less pronounced in the CeM of both males and females. While these studies demonstrated sexual dimorphism in alcohol's acute effects in the CeA, the basis for the more prominent link between stress and alcohol in females remains unknown. We investigated whether the stress hormone corticosterone, which females release at higher levels than males after stress, could modulate the neuronal response to alcohol in the CeA. To investigate sexual dimorphism in corticosterone and alcohol modulation of BLA-CeA activity, we performed intracellular recordings of BLA-eEPSPs in the CeA of male and female rat brain slices. We found that the maximal amplitude of CeL eEPSPs was significantly reduced by 100 nM corticosterone in females, occluding any further significant reduction in eEPSP amplitude upon co-application of 44 mM alcohol. This was not observed in the medial CeA of female rats, where corticosterone treatment did not significantly alter eEPSPs, whereas subsequent addition of alcohol did significantly reduce eEPSPs. Conversely, corticosterone slightly decreased eEPSP amplitude in both CeL and CeM neurons from male rats, although this effect was significant only in the CeM. Subsequent co-application of alcohol significantly reduced eEPSP magnitude in both the CeL and CeM. We found no significant treatment-related changes in paired pulse ratios, suggesting likely postsynaptic sources for the sex differences in corticosterone and alcohol effects. Together these data demonstrate that the female CeL displays great sensitivity to corticosterone, thereby blunting the subsequent response to alcohol. Sexual dimorphism in corticosterone's effects in the CeA implicates corticosterone as a possible moderator of sexual dimorphism in stress-alcohol interactions.

Disclosures: M.L. Logrip: None. **C. Oleata:** None. **M. Roberto:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.12/J8

Topic: C.17. Drugs of Abuse and Addiction

Support: NHMRC ECF 1053308

Title: Ventral subiculum is critical for context-induced relapse to alcohol seeking after punishment-imposed abstinence

Authors: *N. J. MARCHANT[1], K. KAGANOVSKY[1], E. J. CAMPBELL[2], J. M. BOSSERT[1], A. BONCI[1], Y. SHAHAM[1];
[1]Natl. Inst. On Drug Abuse, Baltimore, MD; [2]Sch. of Biomed. Sci. and Pharmacy, Univ. of Newcastle, and Hunter Med. Res. Inst., Newcastle, NSW, Australia

Abstract: Background: Alcoholics typically abstain because of negative consequences associated with excessive drinking and exposure to contexts previously associated with alcohol use often triggers relapse. We recently developed a rat model that captures some characteristics of this human condition: exposure to the alcohol self-administration environment (context A) after punishment-induced suppression of alcohol seeking in a different environment (context B) provokes relapse to alcohol seeking in alcohol-preferring P-rats. Here, we studied the role of ventral subiculum (vSub) and projections from vSub to nucleus accumbens (NAc) shell in this form of relapse. Methods: We first assessed the effect of reversible inactivation of vSub by GABAa+GABAb receptor agonists (muscimol+baclofen) on context-induced relapse to alcohol seeking. We then assessed neuronal activity associated with context-induced relapse by measuring Fos, a marker of neuronal activity. We combined Fos with the retrograde tracer cholera toxin subunit B (CTb, injected into NAc shell), to assess activation in neurons projecting to NAc shell. We assessed activation in glutamatergic inputs to NAc shell, including vSub, ventral medial prefrontal cortex (vmPFC), paraventricular thalamus (PVT), or basolateral amygdala (BLA). Results: Muscimol+baclofen injections into vSub decreased context-induced relapse after suppression of alcohol seeking by punishment. Double-labeling analysis of Fos+CTb demonstrated that context-induced relapse was associated with selective activation of NAc shell projecting neurons in vSub but not vmPFC, PVT, or BLA. Conclusion: These results demonstrate a critical role of vSub in context-induced relapse to alcohol seeking after punishment and suggest that vSub may promote alcohol seeking during relapse by activation of NAc shell.

Disclosures: N.J. Marchant: None. **K. Kaganovsky:** None. **E.J. Campbell:** None. **J.M. Bossert:** None. **A. Bonci:** None. **Y. Shaham:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.13/J9

Topic: C.17. Drugs of Abuse and Addiction

Support: AA020919

DA035958

Title: NMDA and GABAA receptor-mediated plasticity in the ventral tegmental area by acute and chronic ethanol

Authors: *A. NELSON, T. J. WOODWARD, H. PARK, S. I. SHIN, S. S. PISTORIUS, S. D. BAIR, S. C. STEFFENSEN;
Brigham Young Univ., Provo, UT

Abstract: Ventral tegmental area (VTA) GABA neurons are important substrates for alcohol effects in the mesolimbic dopamine (DA) system originating in the VTA and projecting to the nucleus accumbens (NAc). We have previously reported that VTA GABA neurons are sensitive to ethanol at physiologically-relevant doses, exhibit tolerance to acute ethanol, and evince marked hyperactivity during withdrawal from chronic ethanol, which may explain the deficits in DA transmission associated with alcohol dependence. We evaluated glutamate (GLU) NMDA and GABAA receptor-mediated synaptic transmission to VTA GABA neurons during withdrawal from acute and chronic ethanol. To accomplish these studies, we used standard whole-cell and cell-attached mode electrophysiological techniques to evaluate VTA GABA neuron responses in CD-1 GAD GFP mice. In naïve animals, withdrawal from an *in vivo* 24 hr intoxicating dose of ethanol (4 g/kg), enhanced the AMPA/NMDA ratio in VTA GABA neurons. In animals made dependent on ethanol by twice daily injections of 2.5 g/kg ethanol for 6 days, there was no change in the AMPA/NMDA ratio. Similar findings were obtained when animals were exposed to chronic intermittent ethanol (CIE) in alcohol vapor chambers, where they were exposed to 250 mg% blood alcohol levels for 12 hours/day during their dark cycle for 3 weeks. High frequency stimulation induced long-term depression (LTD) of evoked GABAA receptor-mediated IPSCs in VTA GABA neurons in air-exposed animals. Work is in progress to evaluate LTD(GABA) in mice exposed to CIE. These findings suggest that GLU NMDA receptor-mediated plasticity accompanies withdrawal from a single exposure to ethanol, but GABAA receptor-mediated plasticity is operational during withdrawal from chronic exposure to ethanol, suggesting that a switch occurs in GABAA receptor inhibition similar to what has been shown during opiate dependence. These findings have important implications for understanding the adaptations in the mesolimbic reward pathway along the continuum to alcohol dependence.

Disclosures: A. Nelson: None. **T.J. Woodward:** None. **H. Park:** None. **S.I. Shin:** None. **S.S. Pistorius:** None. **S.D. Bair:** None. **S.C. Steffensen:** None.

Poster

314. Alcohol and Stress

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 314.14/J10

Topic: C.17. Drugs of Abuse and Addiction

Support: AA020919

DA035958

Title: Functional switch in GABA(A) receptors on VTA GABA neurons by acute and chronic ethanol

Authors: *H.-J. PARK, A. C. NELSON, T. J. WOODWARD, S. I. SHIN, S. S. PISTORIUS, S. D. BAIR, S. C. STEFFENSEN;
Brigham Young Univ., Provo, UT

Abstract: The motivational effects of opiates and ethanol switch from a dopamine (DA)-independent to a DA-dependent pathway during drug dependence. A corresponding change occurs in ventral tegmental area (VTA) GABA(A) receptors in opiate-dependent animals, which switch from a GABA-induced hyperpolarization of VTA GABA neurons to a GABA-induced depolarization. This effect occurs due to increased BDNF expression and corresponding activation of the trkB receptor on VTA GABA neurons. The aim of this study was to evaluate VTA GABA neuron excitability and GABA synaptic transmission to VTA GABA neurons under ethanol-naïve, acute and dependent conditions. To accomplish these studies, we used standard whole-cell and cell-attached mode electrophysiological techniques to evaluate acute and chronic ethanol effects on VTA GABA neurons in CD-1 GAD GFP mice. In saline-injected controls, superfusion of the GABA(A) receptor agonist muscimol (IC50 = 100 nM) decreased VTA GABA neuron firing rate in a dose-dependent manner. In animals given a single, *in vivo* 24 hr intoxicating dose of ethanol (4.0 g/kg), VTA GABA neuron firing rate was relatively resistant to the effects of muscimol. Similar findings were seen in mice made dependent on ethanol by twice daily injections of 3.0 g/kg ethanol for two weeks or chronic intermittent ethanol (CIE) vapor exposure (200 mg% BAL) for 2-3 weeks. BDNF expression was increased in both the VTA and the nucleus accumbens (NAc) during withdrawal from CIE. Work is in progress to evaluate the effects of blocking the trkB receptor on alcohol dependence and VTA GABA activity. These findings suggest that VTA GABA neurons undergo a switch in GABA(A) receptor function in ethanol-dependent animals, similar to opiate-dependent animals. There also appears to be some GABA(A) plasticity after an acute administration of ethanol. We suggest these changes occur through BDNF activation of the trkB receptor.

Disclosures: H. Park: None. **A.C. Nelson:** None. **T.J. Woodward:** None. **S.I. Shin:** None. **S.S. Pistorius:** None. **S.D. Bair:** None. **S.C. Steffensen:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.01/J11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA15758

Title: Estrogen-potentiated reinstatement of cocaine seeking

Authors: *E. M. DONCHECK[1], J. J. TUSCHER[2], M. C. DEBAKER[1], L. A. URBANIK[1], L. E. MCCARTAN[1], E. E. HERDEMAN[1], K. M. FRICK[2], J. R. MANTSCH[1];
[1]Biomed. Sci., Marquette Univ., Milwaukee, WI; [2]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Although it is generally accepted that peak physiological levels of the ovarian hormone estrogen confer enhanced relapse vulnerability in female cocaine addicts, the underlying mechanisms are not yet well understood. To investigate this, we sought to determine whether the primary estrogen 17β-estradiol (E2), like the stress hormone corticosterone, can promote reinstatement of cocaine seeking in response to a "subthreshold" dose of cocaine that is otherwise insufficient for reinstatement. Sexually mature female Sprague Dawley rats (90 days old/280g minimum at study onset) were surgically implanted with intravenous catheters and underwent short access (2 hour) cocaine self-administration (0.5mg/kg/0.2mL i.v. infusion) for 14 days prior to extinction training. To avoid effects on self-administration and extinction and isolate effects on reinstatement and related responses, rats did not undergo surgical ovariectomy (OVX) until after reaching extinction criterion (<15 lever presses/2-hour session for 2 consecutive days). After allowing 7 days to recover and ensuring that responding still met extinction criterion, a counterbalanced design was used to administer the following tests to each rat: E2 (10ug/kg, i.p.; 1hr pretreatment) + saline, vehicle + saline, E2 + cocaine (various doses, i.p.), vehicle + cocaine. We determined that, under these conditions, both 0.625mg/kg and 1.25mg/kg cocaine were subthreshold doses, while 2.5mg/kg cocaine was a supra-threshold dose. Although pretreatment with E2 had no effect on 0.625mg/kg cocaine, potentiated reinstatement was seen with E2 + 1.25mg/kg cocaine. Furthermore, E2 potentiated responding to 2.5mg/kg cocaine, suggesting a synergistic effect between the hormone and psychostimulant. Although further testing is required to determine if higher physiologically-relevant doses of E2 may potentiate reinstatement, these results indicate that we have developed a model to study how estrogen may set the stage for relapse in female cocaine addicts. Further investigations into localized mechanisms are currently underway.

Disclosures: E.M. Doncheck: None. **J.J. Tuscher:** None. **M.C. DeBaker:** None. **L.A. Urbanik:** None. **L.E. McCartan:** None. **E.E. Herdeman:** None. **K.M. Frick:** None. **J.R. Mantsch:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.02/J12

Topic: C.17. Drugs of Abuse and Addiction

Support: by NIDA grant DA015758 to JRM

Title: Role of a crf receptor-regulated dopaminergic projection from the ventral tegmental area to the prelimbic cortex in stress-induced relapse

Authors: *O. VRANJKOVIC, T. M. KLOEHN, M. E. NORDNESS, D. A. BAKER, J. R. MANTSCH;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Relapse to cocaine use is frequently precipitated by stress. It is well accepted that mesocortical dopamine (DA) neurons originating from the ventral tegmental area (VTA) and projecting into the prelimbic cortex (PL) are involved in drug-seeking behavior. However, the neural mechanism through which stress regulates VTA neurons remains elusive. We and others have implicated the neuropeptide corticotropin releasing factor (CRF) in stress-induced relapse. We hypothesize that CRF release into the VTA during stress activates a subset of VTA DA neurons that project into the PL, resulting in DA D1 receptor activation and, reinstatement of drug-seeking behavior. Here we show that electric footshock (EFS)-induced reinstatement is associated with an increase in PL Fos expression and is observed in animals with a history of long access (LgA; 6hr) but not short-access (ShA; 2hr) self-administration (SA). This is consistent with our previous finding that stress-induced reinstatement is dependent on history of LgA. In addition, using the retrograde tracer, cholera toxin b, we show that VTA cells that project into the PL are active during stress-induced reinstatement. Stress-dependent PL Fos activity is, in part, due to the activation of VTA CRFR1. Antagonism of CRFR1 with intra VTA antalarmin (250ng) prevented the increase in Fos expression within the PL and EFS-induced reinstatement of cocaine seeking. We also examined the role of VTA CRFR1-mediated activation of DA projections to the PL and, thereby, D1 receptor activation in the PL, in stress-induced reinstatement using a disconnection approach involving unilateral intra-VTA delivery of the CRFR1 antagonist, antalarmin and contralateral intra-PL injection of the D1 receptor antagonist, SCH 23390 (200ng). Disconnection of the PL-VTA pathway blocked stress-induced reinstatement of cocaine seeking. Ipsilateral control injections failed to block EFS-induced reinstatement. Thus, the increase in PL Fos is dependent on both a history of LgA SA, and activation of CRFR1 within the VTA, consistent with our previous report that reinstatement to intra-CRF (300ng) VTA is heightened following LgA SA. Heightened CRF-mediated drug seeking and activation of the mesocortical pathway following LgA SA is likely attributable to increased VTA CRFR1 expression, as VTA CRFR1 mRNA expression measured using *in situ*

hybridization is increased in LgA rats relative to ShA rats and saline controls. Our results suggest that VTA CRFR1 activation induces relapse to cocaine use by activating DA cells that project to the PL and that CRFR1 regulation of this pathway is heightened as a result of prior cocaine use.

Disclosures: O. Vranjkovic: None. **T.M. Kloehn:** None. **M.E. Nordness:** None. **D.A. Baker:** None. **J.R. Mantsch:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.03/J13

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA038663

Title: Corticosterone potentiates reinstatement of cocaine seeking through endocannabinoid-mediated inhibition of GABAergic neurotransmission in the prelimbic cortex

Authors: *J. R. MCREYNOLDS[1], E. M. DONCHECK[1], O. VRANJKOVIC[1], E. N. GRAF[1], Q.-S. LIU[2], C. J. HILLARD[2], J. R. MANTSCH[1];
[1]Biomed. Sci., Marquette Univ., Milwaukee, WI; [2]Pharmacol. and Toxicology, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Stress is a powerful trigger for relapse and can not only induce relapse but can potentiate the response to other triggers for drug use. We have shown that under certain self-administration conditions, stress alone does not reinstate cocaine-seeking. However a stressor, electric footshock stress (EFS), can potentiate reinstatement when paired with low dose cocaine. This effect is corticosterone-dependent and the effect of EFS is mimicked by systemic or intra-prelimbic cortex (PL) corticosterone indicating that it is not only necessary but sufficient and the PL, a region critical for reinstatement, is a site of action. Exactly how corticosterone potentiates reinstatement is not fully understood but may involve interactions with the endocannabinoid (eCB) system. Stress increases eCB production in the medial prefrontal cortex, and, like stress-potentiated reinstatement, is glucocorticoid-dependent and we have shown systemically that eCB signaling mediates stress-potentiated reinstatement. The present study examined this effect further by assessing the potential mechanism of corticosterone action in the PL in this effect. Male SD rats self-administered cocaine (0.5 mg/kg/inf; 14 x 2 hrs/day) and then underwent extinction training followed by reinstatement tests. EFS paired with low-dose cocaine- (2.5 mg/kg, ip) induced reinstatement whereas either low dose cocaine or EFS alone did not. Intra-PL infusions of the cannabinoid receptor 1 (CB1R) antagonist, AM251 (0.3 µg) given 15 min prior to reinstatement tests blocked both EFS- and corticosterone-potentiated reinstatement suggesting that these effects are mediated through eCB signaling in the PL. In addition, the effect of EFS or

corticosterone can be mimicked by an intra-PL infusion of the CB1R agonist, WIN 55,212 (50 ng), suggesting that eCB signaling in the PL is not only necessary but sufficient for this effect. The contribution of specific eCBs to potentiated reinstatement is currently being tested. Altogether, these data suggest that glucocorticoid-endocannabinoid interactions in the PL mediate stress-potentiated reinstatement. As CB1Rs are located on GABAergic interneurons in the PL, corticosterone effects may be the result of eCB-mediated inhibition of GABA. In support of this, bath application of corticosterone to PL slices inhibits GABAergic neurotransmission in a CB1R-dependent manner. The involvement of intra-PL GABAergic signaling in corticosterone-potentiated reinstatement is currently being examined. These findings support the hypothesis that corticosterone acts in the PL, through eCB-mediated inhibition of GABA, to potentiate reinstatement of cocaine seeking.

Disclosures: **J.R. McReynolds:** None. **E.M. Doncheck:** None. **O. Vranjkovic:** None. **E.N. Graf:** None. **Q. Liu:** None. **C.J. Hillard:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH grant DA038663. **J.R. Mantsch:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH grant DA038663.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.04/J14

Topic: C.17. Drugs of Abuse and Addiction

Title: Exposure to anabolic androgenic steroids in adolescent male rats triggers changes in susceptibility to cocaine use in adulthood

Authors: *M. POMPILUS, S. SERRANO, J.-R. GESTE, G. SOTO, T. ORTIZ, T. ORTIZ, W. NORZE, C. S. MALDONADO-VLAAR;
Biol., Univ. of Puerto Rico-Rio Piedras, San Juan, PR

Abstract: Anabolic-Androgenic steroids (AAS) are derivatives of the male sexual hormone. Studies have shown that AAS have abuse potential and are implicated in behavior impairments and somatic dysfunction. Furthermore, many elite athletes take AAS in large doses for muscle growth and to enhance their athletic performance. Common misuse of AAS has recently increased in eight 8th grade and 12th grade students. Research has shown that AAS exposure can affect the dopamine brain reward pathway. The aim of this study is to investigate whether early exposure to AAS (nandrolone decaonate) in adolescent male rats potentiate cocaine self-

administration in adulthood and modulates long lasting neurochemical brain changes. We also assessed the role of androgen receptor (AR) within the mesolimbic dopamine system following AAS treatment. Two separate groups of Sprague Dawley adolescent male rats (P28) were treated with subcutaneously injections of either supra-physiologic doses (20mg/kp)/d nandrolone or Sesame oil (vehicle) for 10 consecutive days. At P72 days, animals were implanted with intravenous catheters and trained in cue induced- cocaine seeking behavior paradigm. Our preliminary data demonstrated that pre-exposure to nandrolone during adolescence, tends to increase cocaine self-administration and significantly decrease reinstatement of cocaine seeking behavior in adulthood. In addition, significant changes in the size of several organs were observed. This study suggests that previous use of AAS increases the propensity to cocaine use and morphologic organs changes in adulthood.

Disclosures: M. Pompilus: None. **S. Serrano:** None. **J. Geste:** None. **G. Soto:** None. **T. Ortiz:** None. **T. Ortiz:** None. **W. Norze:** None. **C.S. Maldonado-Vlaar:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.05/J15

Topic: C.17. Drugs of Abuse and Addiction

Support: Commonwealth of Pennsylvania CURE Addiction Center of Excellence

NIDA T-32 Training Fellowship

NIDA U54 Cocaine Cooperative Medication Development Center

Title: A history of physical, emotional, or sexual abuse predicts higher mesolimbic response to drug cues in cocaine-dependent patients

Authors: *P. REGIER[1], Z. A. MONGE[1], J. J. SUH[1], K. JAGANNATHAN[1], Z. WANG[1], J. F. MAGLAND[2], A. TEITELMAN[3], T. R. FRANKLIN[1], R. R. WETHERILL[1], K. YOUNG[1], M. J. GAWRYSIAK[1,4], D. D. LANGLEBEN[1], C. P. O'BRIEN[1], A. R. CHILDRESS[1];
[1]Psychiatry, [2]Radiology, [3]Sch. of Nursing, [4]VA Med. Ctr., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Aims: Previous studies have reported that a history of adverse experiences is associated with higher rates of mental health issues, including addiction. Here, we investigated whether a history of physical, emotional, or sexual abuse in cocaine-dependent patients was associated with greater cocaine cue-triggered activity in nodes of the mesolimbic reward circuitry **Methods:** Treatment-seeking cocaine-dependent patients (n=26) were recruited as part of a 6 month treatment study. Before the start of the study, participants were administered the

Addiction Severity Index (ASI), which contains questions measuring prior abuse (emotional, physical, or sexual). After inpatient stabilization, participants were scanned with event-related blood-oxygen-level-dependent functional MRI during exposure to brief (500 msec) evocative (cocaine, sexual, aversive) vs. neutral cues. Forty-eight images of each cue type were presented in a quasi-random order. Imaging preprocessing (alignment, registration, normalization, smoothing, and motion correction) and first level analysis were conducted within a standard SPM8 pipeline. The responses to the abuse questions were used to create a split (abuse yes - abuse no) for the *cocaine-neutral* cue contrast. **Results:** From the ASI, there were 12 patients that reported a history of abuse and 14 that reported no abuse. As predicted, patients reporting abuse had greater brain activation to the cocaine (vs. neutral) cues in several mesolimbic regions, including the midbrain (VTA), ventral striatum, dorsal striatum, and caudal orbitofrontal cortex to drug cues compared to patients reporting no abuse ($2<t<5$). **Conclusions:** Individuals with adverse life events have been found to be more susceptible to drug addiction. In our study, even though all patients were cocaine dependent, our results provide initial evidence that a history of abuse could have a brain impact (i.e., heightened limbic response to drug cues) that drives drug seeking. Our results highlight heterogeneity within a cocaine-dependent population, indicating the need for individually-tailored treatment. Importantly, to our knowledge, this is the first evidence of a history of abuse on brain vulnerability that is linked to relapse.

Disclosures: **P. Regier:** None. **Z.A. Monge:** None. **J.J. Suh:** None. **K. Jagannathan:** None. **Z. Wang:** None. **J.F. Magland:** None. **A. Teitelman:** None. **T.R. Franklin:** None. **R.R. Wetherill:** None. **K. Young:** None. **M.J. Gawrysiak:** None. **D.D. Langleben:** None. **C.P. O'Brien:** None. **A.R. Childress:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.06/J16

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA031734

Title: Corticotropin releasing factor and dopamine interactions in a heterogeneous ventral tegmental area: How can aversive experiences heighten cocaine self-administration?

Authors: *E. N. HOLLY[1], C. O. BOYSON[2], S. MONTAGUD-ROMERO[3], J. F. DEBOLD[1], K. A. MICZEK[1];
[1]Dept. of Psychology, Tufts Univ., Medford, MA; [2]Dept. of Psychiatry and Behavioral Neurosci., Univ. of Chicago, Chicago, IL; [3]Unidad de Investigación Psicobiología de las Drogodependencias, Dept. de Psicobiología, Univ. de València, Valencia, Spain

Abstract: Intermittent social defeat (on days 1, 4, 7, and 10) escalates later cocaine self-administration. Both rewarding and stressful stimuli increase extracellular dopamine (DA) in ventral tegmental area (VTA) projection targets_the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc). Corticotropin releasing factor (CRF) and its receptors (CRFR1 and CRFR2) are located in the VTA and may influence DA activity during stress, resulting in later heightened vulnerability to addiction. These experiments explore how CRF acts on its receptors both during and after stress to influence mesocorticolimbic DA and cocaine self-administration. *In vivo* microdialysis on d 1 and 10 of social defeat showed phasic CRF release in the VTA. In the rostral VTA, CRF was phasically increased on d 10, but not d 1. In the caudal VTA, CRF was phasically increased on both d 1 and d 10, with greater increase on d 1. Additionally, baseline CRF concentration was increased on d 10 compared to d 1 regardless of probe placement. This CRF release in the VTA affected extracellular DA in both the mPFC and NAc, mediated by CRFR2. Rats were microinjected with a CRFR1 or CRFR2 antagonist into the VTA prior to each defeat, and microdialysis for DA in the mPFC and NAc conducted concurrently on d 1 and 10. Extracellular DA was significantly increased in both regions during both acute and repeated social defeat. On d 1, intra-VTA CRFR2 antagonism prevented the DA increase in the NAc, while not affecting mPFC DA. CRFR1 antagonism had no effect. On d 10, intra-VTA CRFR2 antagonism prevented the DA increase in both the mPFC and NAc, while CRFR1 antagonism still had no effect. Intra-VTA antagonism of both CRFR1 and CRFR2 during each defeat prevented later escalated cocaine self-administration during a 24 h “binge”. CRFR1 antagonism in the caudal, but not rostral, VTA prevented escalated “binge” cocaine self-administration in stressed rats, while the converse was found for CRFR2 antagonism. VTA CRF also played a role in later cocaine seeking. Rats acquired cocaine self-administration and were placed in forced abstinence for 15 d, followed by context-induced reinstatement testing. Previously stressed rats pressed the previous active lever significantly more than controls, which was blocked by both intra-VTA CRFR1 and CRFR2 antagonism. *In vivo* microdialysis found no phasic increase in CRF during reinstatement, although tonic levels were significantly higher in previously stressed rats compared to controls. In conclusion, CRF in the VTA plays a key role in DA function during stress, promoting neuroadaptations driving later increased drug taking and seeking.

Disclosures: E.N. Holly: None. **C.O. Boyson:** None. **S. Montagud-Romero:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.07/J17

Topic: C.17. Drugs of Abuse and Addiction

Support: NIMH R01 NS073574 to J.M.

NIDA R01 031734 to K.M.

Tufts Center for Neuroscience Research grant P30 NS047243

Title: Identification of a novel CRF-VTA microcircuit in the mouse midbrain underlying stress and psychostimulant sensitization

Authors: *K. L. GOBROGGE[1], A. HOOPER[2], E. HOLLY[1], X. HAN[1], J. DEBOLD[1], J. MAGUIRE[2], K. A. MICZEK[1,2];
[1]Psychology, Tufts Univ., Medford, MA; [2]Neurosci., Tufts Med. Sch., Boston, MA

Abstract: Corticotropin releasing factor (CRF) signaling in the ventral tegmental area (VTA) regulates stress-induced psychostimulant self-administration. However, the source of VTA-CRF and the molecular mechanisms underlying this drug-seeking behavior remain unclear. Thus, we used viral-vector-based tract-tracing by stereotaxically infusing an AAV-Flex-ChR2 virus bilaterally in the ventral-posterior-medial (VPM) region of the VTA in CRF-Cre mice. CRF neurons in the lateral hypothalamus (LH) and dorsal raphe nucleus (DRN) projected to and from the paranigral (PN) and parainterfasicular (PIF) subnuclei of the VTA. DRN-VPM CRF dendrites form putative synapses on subsets of dopaminergic (DA-ergic) neurons co-expressing CRF1/2 receptors in the VPM while LH-VPM CRF processes did not. Both of these circuits were activated, evident by c-FOS-immunnoreactivity (-ir), following a single 30-minute restraint or 15-minute social defeat stress. Although this circuit is activated by chronic stress (i.e., 10 days of social defeat), there are fewer synapses double-labeled with PSD95 and VGLUT1. Thirty-minutes of restraint stress increased CRF-ir fibers in the PN/PIF, consistent with microdialysis studies demonstrating CRF release in the VPM of intruder rats experiencing social defeat stress. Specifically activating the PN/PIF using either optogenetics or Gq-DREADD in CRF-Cre mice was sufficient to mimic chronic social defeat stress-induced drug-seeking behavior. Daily 30-minute activation of CRF signaling in the PN/PIF for 10 consecutive days followed by 10-days of rest induced behavioral sensitization to a single intra-peritoneal injection of dextro-amphetamine (d-AMPH, 1.5mg/kg). Together, these data suggest that CRF neurons in the DRN are activated by stress, releasing CRF in the PN/PIF, which modulates behavioral sensitization to d-AMPH. This research was supported by an NIMH R01 research grant NS073574 to J.M., a NIDA grant 031734 to K.M., and the Tufts Center for Neuroscience Research grant P30 NS047243. The authors declare no biomedical, financial, or potential conflicts of interest.

Disclosures: K.L. Gobrogge: None. **A. Hooper:** None. **E. Holly:** None. **X. Han:** None. **J. DeBold:** None. **J. Maguire:** None. **K.A. Miczek:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.08/J18

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 031734

Title: Prevention of escalated cocaine self-administration and cocaine seeking by CRF R1 antagonist in socially defeated mice

Authors: *X. HAN, K. A. PEREZ, A. J. LOTSTEIN, J. F. DEBOLD, K. A. MICZEK;
Dept. of Psychology, Tufts Univ., Medford, MA

Abstract: Social defeat stress can result in escalated cocaine self-administration and cocaine seeking in rodents. Corticotrophin releasing factor (CRF) represents the initial step in the stress cascade that leads to escalation of drug use and drug seeking behaviors. However, the contribution of CRF receptor subtypes to cocaine reinstatement and their role during different phases of stress-induced cocaine self-administration still remains to be defined. The current study examines the effects of CRF R1 antagonist on escalated intravenous cocaine self-administration and cocaine reinstatement as a result of exposure to social defeat stress in mice. (1) The CRF R1 antagonism (CP 376,395, 15mg/kg) given 30 min prior to each social defeat episode prevented stress-induced cocaine self-administration in mice. (2) Administration of CP 376,395 (5 mg/kg and 15 mg/kg) 10 days after the last episode of social stress, dose-dependently reversed the escalation of cocaine intake. (3) In addition, we showed that CP 376,395 administration prior to reinstatement test decreased stress-induced cocaine seeking behaviors. (4) To further explore the role of CRF R1 in specific brain sites, CP 376,395 (0.5 µg/ 0.2 µl and 1 µg/ 0.2 µl) was delivered directly into the ventral tegmental area (VTA) before cocaine self-administration session 10 days after the last stress episode. Intra-VTA antagonism of CRF R1 was sufficient to reverse stress-induced escalated cocaine self-administration. These findings suggest that CRF R1 exerts multiple roles during initial reaction to social stress and in long-term neuroadaptations that are relevant to escalated cocaine self-administration and cocaine seeking, providing a potential target for therapeutic inventions for stress-induced drug use disorders.

Disclosures: X. Han: None. **K.A. Perez:** None. **A.J. Lotstein:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.09/J19

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA015758

NIH Grant DA0170751

Title: Increased sensitivity to cocaine-induced relapse to drug-seeking behavior in organic cation transporter 3 knockout mice

Authors: *P. J. GASSER, J. R. MCREYNOLDS, O. VRANJKOVIC, O. DERRICKS, B. NINO, T. AMBROSIUS, J. R. MANTSCH;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Cocaine addicts report that craving responses to drug-associated stimuli are intensified during periods of stress, resulting in heightened susceptibility to relapse of drug use. These reports are paralleled by findings that stress can potentiate the reinstatement of cocaine-seeking behavior by drug-associated cues in rodents. Together, these studies suggest that stress may act as a "stage-setter", inducing state-dependent changes in the sensitivity of brain reward circuits to the reinforcing properties of drugs, and enhancing the potency of drugs of abuse or drug-associated cues to induce relapse. Thus, interactions between pathways activated by stress and by cocaine-associated stimuli are likely to be critical determinants of relapse vulnerability. However, the mechanisms underlying these interactions are not completely understood. We recently demonstrated that corticosterone (CORT) acutely blocks dopamine clearance in the nucleus accumbens (NAc), likely mediated by the uptake2 transporter organic cation transporter 3 (OCT3), a high-capacity corticosteroid-sensitive monoamine transporter. We provided evidence that, through this mechanism, CORT potentiates the actions of cocaine on dopamine signaling and reinstatement of drug-seeking behavior in rats. We have hypothesized that decreased clearance of dopamine in the NAc, due to CORT-induced inhibition of OCT3, enhances dopaminergic neurotransmission, resulting in increased sensitivity to natural and cocaine reward, and heightened vulnerability to relapse of cocaine-seeking behavior. While OCT3 is the most likely mechanism underlying CORT effects on dopamine clearance and relapse, support for its role in these processes is based on pharmacological tools (CORT and normetanephrine) which can exert actions at other, non-OCT3, targets. To more definitively test the hypothesis that OCT3 inhibition underlies CORT-induced potentiation of relapse, the present studies examined the effects of CORT and normetanephrine, two inhibitors of OCT3, on cocaine-primed reinstatement of conditioned place preference in wild type and OCT3 knockout mice. Compared to wild-type mice, OCT3-knockout mice were more sensitive to low-dose cocaine-induced reinstatement of conditioned place preference. In wild-type mice, both CORT and normetanephrine potentiated low-dose cocaine-induced potentiation of CPP, while both OCT3 inhibitors were without effect in OCT3 knockout mice. These studies suggest that the previously-described effects of CORT on relapse to drug seeking behavior are mediated, at least in part, by inhibition of OCT3-mediated dopamine transport.

Disclosures: P.J. Gasser: None. **J.R. McReynolds:** None. **O. Vranjkovic:** None. **O. Derricks:** None. **B. Nino:** None. **T. Ambrosius:** None. **J.R. Mantsch:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.10/J20

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA070751 to PJG

Title: Corticosterone potentiates the effect of cocaine on nucleus accumbens dopamine release and clearance

Authors: *D. S. WHEELER, A. L. EBBEN, A. T. BOHN, I. A. JASEK, D. A. BAKER, J. R. MANTSCH, R. A. WHEELER, P. J. GASSER;
Marquette Univ., Milwaukee, WI

Abstract: Stress and cocaine act synergistically to increase extracellular dopamine and drive cocaine seeking. This interaction is mediated in part by the effects of corticosterone in brain areas that receive dopamine innervation, such as the nucleus accumbens and medial prefrontal cortex. We have recently demonstrated that corticosterone acts to decrease dopamine clearance in the nucleus accumbens via a dopamine transporter (DAT)-independent mechanism likely involving the inhibition of organic cation transporter 3 (OCT3)-mediated transport. We have also demonstrated that corticosterone potentiates the effects of cocaine on nucleus accumbens dopamine signaling and drug-seeking behavior. However, it is not known whether the corticosterone-induced reduction in clearance is accompanied by an increase in dopamine release events, or whether corticosterone affects clearance in the absence of DAT blockade. We examined the effect of acute corticosterone treatment on dopamine release and clearance in the nucleus accumbens core and shell of behaving rats to examine how the stress hormone acts centrally to augment the dopamine response to cocaine. Using fast scan-cyclic voltammetry, we measured naturally-occurring transient dopamine release events during a baseline period, after a systemic injection of corticosterone (2 mg/Kg, ip) or vehicle (2.5% EtOH, ip), and after a subsequent systemic injection of low-dose cocaine (2.5 mg/Kg, ip). Results show that corticosterone potentiates cocaine's effect on dopamine transient frequency and amplitude, suggesting that the reduction in dopamine clearance increases the number of spontaneous release events. Furthermore, preliminary findings indicate that corticosterone alone produces a mild increase in extracellular dopamine, suggesting that the inhibition of OCT3 modulates dopamine signaling in the absence of DAT blockade. Ongoing studies are investigating the potential impact of corticosterone on phasic dopamine encoding of natural rewards in order to determine whether the effects of corticosterone extend beyond situations in which the DAT is blocked by cocaine.

Disclosures: D.S. Wheeler: None. **A.L. Ebben:** None. **A.T. Bohn:** None. **I.A. Jasek:** None. **D.A. Baker:** None. **J.R. Mantsch:** None. **R.A. Wheeler:** None. **P.J. Gasser:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.11/J21

Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC Discovery grant

CRC program

Title: Effects of cannabidiol on cocaine self-administration and cue-induced cocaine seeking in male rats

Authors: A. MAHMUD, S. GALLANT, T. D'CUNHA, *U. SHALEV;
Concordia Univ., Montreal, QC, Canada

Abstract: Drug abuse, a chronic disorder involving uncontrollable drug seeking, abstinence, and relapse, causes 47,000 deaths in Canada each year. For cocaine, a psychostimulant drug, a staggering 61.9% of abstinent users relapse and deaths related to its use have increased by 29% from 2001 to 2013. Despite this, adequate treatment has yet to be developed. Recent research in rats demonstrated that cannabidiol (CBD), one of the non-psychoactive compounds in cannabis, attenuated cue-induced heroin-seeking 24 hours after injection, thus suggesting that CBD may be a potential treatment option for opiate use. CBD has also been shown to enhance the extinction of cocaine-induced conditioned place preference. Here, we investigated the effects of CBD on cocaine self-administration and cue-induced cocaine seeking. In experiments 1 and 2, rats were trained to self-administer cocaine in operant chambers and then exposed to a 14 days withdrawal period while housed in the animal colony. Exp. 1: To investigate the effects of CBD on cocaine self-administration, rats were trained to self-administer for 5 days on a fixed ratio 1 (FR-1), followed by 12 days of a progressive ratio schedule of reinforcement. Rats were then injected with three doses of CBD (0.0 mg/kg, 5.0 mg/kg, and 10 mg/kg; i.p.) 30 min before the self-administration session. Exp. 2: To investigate the effects of CBD on cue-induced cocaine seeking, rats were trained on FR-1 schedule for 10 days. Following a 14 day withdrawal period, rats were returned to the operant conditioning chambers and tested for cocaine seeking under extinction conditions. Rats received one of three doses (0.0 mg/kg, 5 mg/kg, or 10 mg/kg), 24 hours prior to the test. Exp. 3: As CBD has been shown to have anxiolytic properties, the efficacy of CBD treatment (0.0 mg/kg and 10 mg/kg) was evaluated using an elevated plus-maze anxiety test. CBD did not attenuate cocaine self-administration or cue-induced cocaine seeking following prolonged withdrawal. In fact, CBD treatment appeared to augment cue-induced cocaine seeking and could thus be a risk factor in cocaine relapse. CBD treatment produced a clear anxiolytic effect in the elevated plus-maze, validating the efficacy of CBD as used in the present experiments. Overall, our findings suggest that CBD may not be a potential treatment option for cocaine addiction. Future studies should investigate the effects of repeated administration and higher doses of CBD, as well as the effects of CBD on cocaine seeking induced by re-exposure to cocaine (priming) or exposure to stress.

Disclosures: **A. Mahmud:** None. **S. Gallant:** None. **T. D'Cunha:** None. **U. Shalev:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.12/J22

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA022340 to JFC

Title: Cannabinoid exposure in adolescence modulates cocaine reward in adulthood

Authors: *J. M. WENZEL[1], J. F. CHEER[1,2];
[1]Anat. & Neurobio., [2]Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Marijuana is the most commonly abused illicit drug among adolescents, and regular use of cannabinoids (CBs) in this vulnerable population is associated with the development of psychiatric disease including drug abuse. Human research indicates that adolescent CB use predicts future cocaine (COC) abuse, however, the underlying cause of this phenomenon is unclear. Adolescent CB exposure disrupts the dopaminergic response to COC in adulthood suggesting that CB use may disturb the subjective experience of COC. It is well documented that immediate euphoria produced by COC administration is subsequently replaced by feelings of dysphoria and anxiety. Thus, it is likely that an individual's experience of either of these opposing processes may motivate successive COC use. Here we utilize a modified place conditioning test to assess how CB exposure in adolescence affects COC's dual positive and negative effects. In this test rats are conditioned to associate a unique environment with the effects of COC present either immediately or 15 min after IV COC injection. Characteristically following place conditioning rats exhibit a preference for the environment paired with COC's immediate/positive effects (a CPP), and an aversion for the environment paired with COC's delayed/negative effects (a CPA). Therefore, we assessed the impact of CB exposure in adolescence on adult COC reward and aversion. Adolescent male rats were treated with one of three doses of the synthetic CB WIN 55,212-2 (WIN; 0.5mg/kg, 2mg/kg, 5mg/kg) or its vehicle (VEH) once per day for eight days (PND35-42). Following treatment rats were left in their home cages until they reached adulthood at which point they underwent place conditioning for either the immediate/positive or delayed/negative effects of COC (PND77-86). Interestingly, while rats treated with VEH during adolescence developed the typical pattern of COC CPP and CPA, exposure to WIN during adolescence dose-dependently resulted in the development of CPA for the immediate effects of COC (an outcome opposite to the canonical COC CPP), while not disrupting CPA for COC's delayed/negative effects. These data suggest that CB exposure in adolescence diminishes COC's rewarding effects in adulthood and, in fact, results in a predominantly negative experience within the first 5 min after administration (i.e. the length of

each conditioning trial). It remains unclear how these alterations in COC reward translate to COC seeking in adulthood. In animal models, however, reduced COC reward is associated with increased COC intake, suggesting that these observed decrements in COC reward might contribute to increased COC administration.

Disclosures: J.M. Wenzel: None. **J.F. Cheer:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.13/J23

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant MH-97988

NARSAD

NIH NCRR P20RR16435

University of Vermont College of Arts and Sciences

Title: Pituitary adenylate cyclase activating polypeptide (PACAP) increases neuronal excitability in the bed nucleus of the stria terminalis (BNST)

Authors: *K. R. LEZAK[1], V. MAY[2], S. E. HAMMACK[1];
[1]Psychological Sci., [2]Neurolog. Sci., Univ. of Vermont, Burlington, VT

Abstract: The bed nucleus of the stria terminalis (BNST) has been implicated in mediating many of the consequences of exposure to stressful stimuli. Pituitary adenylate cyclase- activating polypeptide (PACAP) and its cognate PAC1 receptor have been associated with several mental health disorders that are related to stressor exposure and/or the dysregulation of the HPA axis, including mood and anxiety disorders. We have previously demonstrated increased PACAP and PAC1 receptor expression in the BNST following stressor exposure, and increased BNST PACAP signaling is necessary and sufficient for many of the behavioral and endocrine consequences of stressor exposure. These effects may be, in part, mediated by excitatory effects of PACAP on BNST neurons. In the present studies, we used whole-cell patch-clamp electrophysiological techniques in BNST slices to demonstrate that exogenous PACAP application enhances excitability in a subset of BNST neurons in a manner consistent with an enhancement of the hyperpolarization activated cation current, I_h. BNST neurons have been previously characterized into several physiological cell types; the PACAP response occurred primarily in type 2 neurons, which are characterized by the presence of both I_h and the low-threshold calcium current, I_T. The BNST response to PACAP was blocked by the I_h channel blocker, ZD7288, but not the I_T channel blocker, $NiCl_2$, suggesting that PACAP-induced

excitability was mediated by I_h, but not I_T, enhancement. These results support converging data suggesting that BNST PACAP signaling plays a key role in regulating stress and anxiety-like responding, and maladaptations in BNST PACAP systems may lead stress- related psychopathologies.

Disclosures: K.R. Lezak: None. **V. May:** None. **S.E. Hammack:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.14/J24

Topic: C.17. Drugs of Abuse and Addiction

Support: DA Grant 033123

MH Grant 097988

Title: Intra-bed nucleus of the stria terminalis (BNST)pituitary adenylate cyclase activating peptide (PACAP)infusion reinstates cocaine seeking in rats

Authors: *O. MILES[1], E. A. THRAILKILL[1], V. MAY[2], M. E. BOUTON[1], S. E. HAMMACK[1];
[2]Dept. of Neurolog. Sci., [1]The Univ. of Vermont, Burlington, VT

Abstract: The tendency of users to relapse severely hinders adequate treatment of addiction. Physical and psychological stressors often contribute to difficulties in maintaining behavior change, and may play a significant role in relapse. We have previously shown that the activation of pituitary adenylate cyclase activating peptide (PACAP) systems in the bed nucleus of the stria terminalis (BNST) mediate many consequences of chronic stressor exposure. Hence, chronic stress substantially increased BNST PACAP levels, intra-BNST PACAP infusions produced the behavioral and endocrine consequences of stressor exposure, and BNST PACAP antagonism blocked many of the consequences of chronic stress. In the present set of studies, we investigated the role of BNST PACAP in stress-induced reinstatement of cocaine seeking. All rats self-administered cocaine (3mg/ml; 0.5mg/kg/infusion, i.v.) for 1hr daily over 10 days followed by extinction training in which lever pressing no longer resulted in cocaine delivery. In the first experiment we showed that intra-BNST PACAP infusion (1 μg; 0.5 μl per side) could reinstate previously extinguished cocaine seeking behavior. In the second experiment we found that intra-BNST infusions of the PAC1/VPAC2 antagonist, PACAP 6-38 (1 μg; 0.5 μl per side) blocked reinstatement following stressor exposure (5 sec 2mA footshock). Overall, these data suggest that BNST PACAP systems mediate stress-induced reinstatement to drug seeking. Understanding the neuropharmacology of BNST PACAP in stress-induced reinstatement and the role of PACAP systems may lead to viable targets for relapse prevention.

Disclosures: **O. Miles:** None. **E.A. Thrailkill:** None. **V. May:** None. **M.E. Bouton:** None. **S.E. Hammack:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.15/J25

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA-033370

Title: Antagonism of dopamine D4 receptors in the lateral habenula reduces the anxiogenic response to cocaine in a runway model of drug self-administration

Authors: ***K. SHELTON**[1], K. BOGYO[2], T. SCHICK[2], A. ETTENBERG[1];
[1]Dept. of Psychological and Brain Sci., [2]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Human users of cocaine report that the initial "high", or period of euphoria, that results from taking the drug is temporally replaced by an aversive "crash" that is characterized as a period of agitation, anxiety and anhedonia. Our laboratory has previously reported that self-administered cocaine in rats produces behavioral effects consistent with the human report - that is, an initial experience of reward followed by a period of anxiety and anhedonia. While the positive motivating effects of cocaine have long been thought to require an intact mesolimbic dopamine (DA) system, the neural mechanisms that give rise to the negative effects of the drug remain less clearly defined. Recent literature points to the lateral habenula (LHb) as a site for the encoding of aversive or anxiogenic events and has also been shown to "gate" the activity of the DA reward system by inhibiting the activity of DA cells within the VTA. This "gating" is hypothesized to stem from a negative feedback loop originating in the VTA, and involving DA projections to the LHb and direct and indirect reciprocal connections back to the VTA. The current study investigated the putative modulatory effects of DA stimulation on the aversive/anxiogenic properties of cocaine as measured in a runway model of IV self-administration. Male rats were stereotaxically implanted with bilateral cannulae aimed at the LHb and then trained to run a straight alley for IV cocaine (1.0 mg/kg) delivered upon arrival in a goal-box. As we have previously reported, vehicle pretreated controls developed approach-avoidance conflict behaviors about goal-box entry that are reflective of the dual positive and negative effects of IV cocaine. The frequency of such behaviors was significantly diminished in animals pretreated with bilateral intra-LHb injection of the D4 receptor antagonist, L-745,870. These results suggest that DA neurons in the VTA may not only be involved in producing the positive-rewarding effects of cocaine, but also actively subduing the negative after-affects of the drug. This work was supported by NIDA grant DA-033370 awarded to AE.

Disclosures: **K. Shelton:** None. **K. Bogyo:** None. **T. Schick:** None. **A. Ettenberg:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.16/J26

Topic: C.17. Drugs of Abuse and Addiction

Support: DA-033370

Title: Activation of serotonin 1B autoreceptors in the Bed Nucleus of the Stria Terminalis attenuates the negative/anxiogenic effects of cocaine

Authors: ***A. KLEIN**, M. BRITO, N. LE, T. OHANA, A. PATIL, C. PROVENZANO, A. WEI, A. ETTENBERG;
Psychological and Brain Sci., UC Santa Barbara, Santa Barbara, CA

Abstract: Consistent with the Opponent Process Theory of motivated behavior, cocaine administration produces dual and opposing affective states: an initial rewarding "high" followed by a dysphoric/anxiogenic "crash". It therefore seems likely that the motivation to seek cocaine is dependent upon the organism's assessment of the positive relative to the negative consequences of its use. While the neurobiology of the reinforcing aspects of cocaine has been well established, less is known about the systems responsible for the drug's negative actions. In this context, the current study involved an assessment of serotonergic (5-HT) function, which is enhanced by cocaine administration and has been linked to the presence of anxiogenic and depressive states in human and animal studies. In particular, we investigated the role of 5-HT within the bed nucleus of the stria terminalis (BNST) - a structure within the extended amygdala that is activated during periods of stress and during the negative affective state associated with the withdrawal from drugs of abuse. The present study tested the hypothesis that increased 5-HT release in the BNST contributes to the anxiogenic effects of cocaine. A runway self-administration paradigm was employed in which animals were trained to traverse a straight alley in order to earn an infusion of IV cocaine (1.0mg/kg) delivered upon goal box entry. Testing consisted of 16 single daily trials. In this task, animals develop ambivalence about goal box entry (reflected by the development of approach/avoidance "retreat" behaviors) that we have shown to reflect the dual positive (rewarding) and negative (anxiogenic) associations that subjects form with the cocaine-paired goal-box. To assess the involvement of 5-HT signaling within the BNST on this conflict behavior, prior to each trial rats received bilateral intracranial injections of CP94,253 (0.0µg, 0.5µg, or 1.0µg/side in 0.5/µl), a potent and selective 5-HT1B agonist that inhibits local 5-HT release via activation of terminal autoreceptors. Results indicated that CP94,253 did not alter the positive incentive properties of cocaine (start latencies were unaffected) nor did it alter gross motor behavior (as revealed in subsequent locomotor activity

testing). Treatments did, however, selectively attenuate the negative effects of cocaine, as indicated by a dose-dependent decrease in the frequency of approach-avoidance "retreat" behaviors. We therefore conclude that 5-HT signaling within the BNST likely contributes to the negative/anxiogenic effects of cocaine. This work supported by NIDA grant DA-033370 awarded to AE.

Disclosures: **A. Klein:** None. **M. Brito:** None. **N. Le:** None. **T. Ohana:** None. **A. Patil:** None. **C. Provenzano:** None. **A. Wei:** None. **A. Ettenberg:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.17/J27

Topic: C.17. Drugs of Abuse and Addiction

Title: Role of anterior dorsal lateral hypothalamic area perineuronal nets in the acquisition of cocaine-induced conditioned place preference

Authors: ***J. M. BLACKTOP**[1], L. CHURCHILL[2], R. P. TODD[1], M. SLAKER[1], B. A. SORG[1]; [1]Dept. of Integrative Physiol. and Neurosci., Washington State Univ. Vancouver, Vancouver, WA; [2]Dept. of Integrative Physiol. and Neurosci. (IPN), Washington State Univ., Pullman, WA

Abstract: Addiction involves drug-induced neuroplasticity of the circuitry of motivated behavior, which includes the medial forebrain bundle and the lateral hypothalamic area. Emerging at the forefront of neuroplasticity regulation are specialized extracellular matrix structures that form perineuronal nets (PNNs) around certain neurons, mainly parvalbumin positive (PV+) fast-spiking interneurons (FSINs), making them a promising target for the regulation of drug-induced neuroplasticity. Despite the emerging significance of PNNs in drug-induced neuroplasticity and the well-established role of the lateral hypothalamic area (LHA) in reward/reinforcement/motivation, very little is known about how PNN-expressing neurons control drug-seeking behavior. The goals of this experiment were: 1) to determine areas of high PNN expression within the LHA, and 2) whether PNN expression within the LHA is necessary for the rewarding effects of cocaine exposure, measured by conditioned place preference (CPP). A discrete region of the anterior dorsal LHA (LHAad) was found to exhibit robust PNN expression, while the anterior ventral LHA (LHAav) exhibited comparatively sparse PNN expression. Compellingly, PNN removal via chrondroitinase ABC (Ch-ABC) administration in the dorsal but not the ventral anterior LHA prior to conditioning abolished acquisition of cocaine-induced conditioned place preference, highlighting the importance and specificity of PNN removal within this subregion of the LHA. Consistent with previous findings, it was determined approximately that 87% of WFA positive neurons co-expressed parvalbumin in the LHAad of drug naïve rats. Additionally, removal of PNNs in the LHAad did not affect total

locomotor activity or high-fat food intake in a separate group of cocaine naïve animals. In summary, preliminary data indicate that PNN expression in the LHAad: 1) is necessary for acquisition of cocaine-induced CPP, 2) is predominantly co-localized with parvalbumin, and 3) is not necessary for normal locomotor or ingestive behavior.

Disclosures: J.M. Blacktop: None. **L. Churchill:** None. **R.P. Todd:** None. **M. Slaker:** None. **B.A. Sorg:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.18/J28

Topic: C.17. Drugs of Abuse and Addiction

Support: VA Grant 589-KG-0012

NIH Grant R21-DA029787

Title: Fos and dopamine activation in reward pathways of rats selectively bred for enhanced drug self-administration

Authors: *H. XU, S. HE, K. GRASING;
Lab. of Substance Abuse, Kansas City VA Med. Ctr., Kansas City, MO

Abstract: Background: Vulnerability to psychostimulants varies greatly among different individuals, with genetic factors contributing to these differences. In one comparative analysis of humans with substance abuse disorders, heritability was highest for cocaine relative to other forms of drug abuse.The LS and HS rat lives were developed by selective breeding for low and high levels of intravenous drug self-administration in our laboratory. For low-dose cocaine, HS rats self-administer approximately five-fold more injections than LS animals. Here, we explored cocaine-induced dopaminergic activation in brain reward circuitry of LS and HS animals. Methods: Conditioned-place preference (CPP) testing was performed in three-chamber shuttle boxes using an un-biased procedure. Rats were conditioned with low dose (daily intraperitoneal injections of 0.7, 1.3, 2.7, and 5.3 mg/kg) or high dose (daily injections of 2.0, 4.0, 8.0, and 16.0 mg/kg) cocaine under an ascending-dose protocol for CPP, with cocaine preference tested three days after conditioning. Subsequently, animals were euthanized and brains examined for c-fos, dopamine d1 (D1R) as well as D2 (D2R) receptor activation in nucleus accumbens core (NAc) and shell (NASh), caudate putamen (CPu), ventral tegmental area (VTA) and dentate gyrus (DG) by immunofluorescent-staining analysis. Results: Both low and high dose cocaine induced CPP in both strains. Preferences were significantly larger in HS animals, with no significant interaction between strain and cocaine dose. Relative to the LS strain, both cocaine doses produced greater c-fos expression and activation of D1R and D2R neurons in HS rats, for all five

brain regions. Compared to high-dose cocaine, D2R activation was significantly elevated in HS rats that received low-dose cocaine, but not LS animals. A similar of pattern of increased D1R activation for low-dose treated HS rats was observed in NASh, CPu, and VTA only. Conclusion: HS rats exhibit greater cocaine-induced CPP, fos activation, and activation of D1R and D2R relative to LS animals in various brain regions including the NAc, NASh, CPu, VTA and DG. HS rats are more sensitive to drug-induced activation of the dopamine system by low-dose cocaine in a subset of brain reward regions. This mechanism may underlie their enhanced sensitivity to cocaine.

Disclosures: **H. Xu:** None. **S. He:** None. **K. Grasing:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.19/J29

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA016511

Title: Extended cocaine-seeking produces a shift from goal-directed to habitual responding in rats

Authors: *K.-C. LEONG, C. R. BERINI, S. M. GHEE, C. M. REICHEL;
Dept. Of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Cocaine addiction is often characterized by a rigid pattern of behaviors in which cocaine users continue seeking and taking drug despite negative consequences associated with its use. As such, full acquisition and relapse of drug-seeking behavior may be attributed to a shift away from goal-directed responding and a shift towards the maladaptive formation of rigid and habit-like responses. This rigid nature of habitual responding is typically characterized by insensitivity to changes in outcome value, which can be developed with extended training. Rats given extended access to self-administered cocaine are considered to have transitioned from a recreational or limited drug access to chronic use patterns indicated by an escalation of intake and higher reinstated behaviors. Additionally, extended access protocols offer an extensive number of drug trials that provides a nice framework to study drug-seeking habits following transition from recreational to chronic drug use. The present study determined whether cocaine (primary reinforcer) and cocaine associated cues (secondary reinforcer) could be devalued in rats with different histories of cocaine self-administration. All rats received one-hour cocaine self-administration sessions for 7 days, followed by either 14 days of six-hour sessions (long-access) or 14 days of continued one-hour sessions (short-access). Following acquisition, rats received outcome devaluation before undergoing a 7 day period of abstinence. The paired group received

cocaine (administered i.v. through a playback program based on the number of infusions received on the last day of self-administration) immediately followed by an aversive compound lithium chloride (LiCl; 0.6 M, 5 ml/kg, i.p.) before being placed into a holding cage. The unpaired group received LiCl injections 6 hours prior to cocaine infusions. Rats were given two reinstatement tests, the first in which they were exposed to the cocaine-paired cues only and the second in which rats were exposed to cocaine itself via a contingent lever response. Cocaine history did not have an impact on devaluation of cocaine-associated cues. However, only rats on a short-access cocaine schedule displayed devaluation to the reinforcing properties of cocaine, but rats trained on a long-access schedule did not. Taken together this pattern of results suggests that, in short access rats, devaluation is specific to the primary reinforcer and not associative stimuli such as cues. Importantly, rats that received extended training during self-administration displayed insensitivity to outcome devaluation of the primary reinforcer as well as all associative stimuli.

Disclosures: **K. Leong:** None. **C.R. Berini:** None. **S.M. Ghee:** None. **C.M. Reichel:** None.

Poster

315. Cocaine: Mechanisms of Reinforcement and Relapse

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 315.20/J30

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant K99DA038110

NIH Grant DA009621

NIH Grant DA015835

Title: Chronic stress exposure during early withdrawal from extended access cocaine self-administration facilitates incubation of cue-induced cocaine craving

Authors: ***J. A. LOWETH**[1], R. M. GLYNN[1], J. A. ROSENKRANZ[2], M. E. WOLF[1]; [1]Neurosci., [2]Cell. and Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: A major challenge for treating cocaine addiction is the propensity for abstinent users to relapse. Two important triggers for relapse are cues associated with prior drug use and stressful life events. Human studies indicate that exposure to chronic adverse life events is associated with increased relapse vulnerability, indicating a need for animal models that explore interactions between chronic stress and drug withdrawal. However, the majority of studies investigating stress-induced relapse vulnerability have examined the effects of acute stressors on the reinstatement of previously extinguished drug seeking behavior, a model which may not

accurately depict the situation of addicts, who typically do not undergo extinction training and may relapse after a long drug-free period. To study the effect of chronic stress on withdrawal-dependent changes in relapse vulnerability, we used the incubation model of craving and relapse in which cue-induced drug seeking progressively intensifies ("incubates") during withdrawal from extended-access cocaine self-administration. Food restriction or repeated restraint stress were used as chronic stressors. Rats self-administered cocaine under extended-access conditions (6 h/d for 10 d) that have been shown to produce incubation of craving. On the day after the last self-administration session [withdrawal day (WD) 1], rats received a test for cue-induced cocaine seeking, during which nose-pokes resulted in presentation of the light cue but not cocaine. Rats were then divided into 2 groups destined for either control or stress conditions. In the food restriction studies, rats underwent a 2 week period of mild, chronic food restriction stress starting on WD2 (body weight maintained at 90% of their baseline weight). Control rats had ad libitum access to food. On WD15, rats underwent a second seeking test. In the repeated restraint studies, rats underwent 7 daily restraint sessions (20 min) over a 9 day period from WD6 to WD14 and received a seeking test on WD15, a day after the last repeated restraint session. Controls were placed in a cage with bedding on the same schedule. As expected, we found that controls showed greater cue-induced cocaine seeking on WD15 compared to WD1 (i.e. incubation of craving). Interestingly, rats in both stress groups showed a more robust increase in seeking on WD15, indicating acceleration or facilitation of incubation. Separate studies showed that the enhanced cocaine seeking observed was due to chronic and not acute stress. These data indicate that chronic stress during early withdrawal facilitates incubation of cocaine craving, which is thought to contribute to enhanced relapse vulnerability.

Disclosures: J.A. Loweth: None. **R.M. Glynn:** None. **J.A. Rosenkranz:** None. **M.E. Wolf:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.01/J31

Topic: C.17. Drugs of Abuse and Addiction

Support: This work is supported by the Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

Title: Rats that experience footshock-induced abstinence from methamphetamine self-administration exhibit increased prodynorphin mRNA in the nucleus accumbens

Authors: *M. T. MCCOY, O. V. TORRES, B. LADENHEIM, I. N. KRASNOVA, J. CADET; Mol. Neuropsychiatry Res. Br., DHHS/NIH/NIDA/IRP, Baltimore, MD

Abstract: Methamphetamine (METH) is an illicit psychostimulant that is abused worldwide. Despite the longterm problem of METH addiction, cellular and molecular mechanisms involved in the transition from occasional to habitual drug use remain to be elucidated. Towards this end, our laboratory has investigated the effects of METH self-administration (SA) on the expression of neuroplasticity-associated genes within the dorsal striatum. Here we examined the influence of footshock on METH SA. We also measured potential changes on gene expression in the nucleus accumbens (NAc) of rats self-administering METH. Male Sprague-Dawley rats were trained to self-administer METH (0.1 mg/kg/injection, i.v.) or saline during twenty-two (9-hr drug access) sessions. After that time, foot-shocks were administered in increasing intensity over a period of thirteen sessions. The rats were then tested for cue-induced drug craving at 2 and 21 days post-shock and euthanized 9 days after the second extinction test. We extracted RNA from the nucleus accumbens (NAc), made cDNA, and ran quantitative polymerase chain reaction (PCR). Foot-shock caused the separation into two distinct SA groups: shock-resistant (SR) rats that continued to press the lever for METH despite the negative consequence and the shock-sensitive (SS) rats that significantly reduce their lever pressing. Our PCR results reveal that the SS group had a significantly increased expression of prodynorphin, but not of proenkephalin, mRNA levels, relative to the rats in the control and the SR groups. The increase expression of this neuropeptide in the SS group suggests that the two groups of rats that respond differently to footshocks also differ in their expression of this kappa receptor agonist. Our data suggest that this model of METH SA with adverse consequences may provide greater insight into the mechanisms of relapse to METH in clinical situations. Acknowledgement: This work is supported by the Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

Disclosures: M.T. McCoy: None. **O.V. Torres:** None. **B. Ladenheim:** None. **I.N. Krasnova:** None. **J. Cadet:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.02/J32

Topic: C.17. Drugs of Abuse and Addiction

Title: Phentermine induces the conditioned rewarding effects via the activation of PI3K/Akt signaling pathway in the nucleus accumbens

Authors: *S.-I. HONG, S.-X. MA, J.-Y. HWANG, J.-Y. SEO, Y.-H. KO, S.-Y. LEE, C.-G. JANG;
Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Phentermine is similar to methamphetamine in structure and the most widely used anti-obesity drug in the United States. Phentermine has been raised as an abuse potential, however, the mechanism of phentermine dependence has not been established. The aim of this study was to investigate whether phentermine produces the conditioned rewarding effects via the activation of PI3K/Akt signaling pathway in nucleus accumbens (NAc) of mice. In this study, we investigated the abuse liability of phentermine by using conditioned place preference (CPP) and climbing behavior in mice. In addition, we observed changes in expression of DAT and phosphorylation of Akt in NAc. As a result, phentermine 3 mg/kg significantly increased CPP and climbing behavior in mice. Moreover, repeated treatment with phentermine significantly increased expression of DAT protein and phosphorylation of Akt in the NAc. Furthermore, we examined whether LY294002, a specific PI3K/Akt inhibitor, could block phentermine-induced CPP and climbing behavior in mice. LY294002 (3 μg/site, i.c.v.) not only reduced CPP and climbing behavior but also decreased expression of DAT protein and phosphorylation of Akt in the NAc. These findings suggest that phentermine induces the conditioned rewarding effects via the activation of PI3K/Akt signaling pathway in NAc.

Disclosures: **S. Hong:** None. **S. Ma:** None. **J. Hwang:** None. **J. Seo:** None. **Y. Ko:** None. **S. Lee:** None. **C. Jang:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.03/J33

Topic: C.17. Drugs of Abuse and Addiction

Support: Scientific Research on Innovative Areas of the Ministry of Education, Culture, Sports, Science and Technology, JAPAN

The Japan China Medical Association

Research Fellow of Japan Society for the Promotion of Science

Title: BDNF-TrkB signaling in the nucleus accumbens plays a key role in methamphetamine withdrawal symptoms

Authors: *Q. REN, M. MA, C. YANG, J.-C. ZHANG, W. YAO, K. HASHIMOTO;
Chiba Univ. Ctr. Forensic Mental Hlth., Chiba, Japan

Abstract: Depression is a core symptom of methamphetamine (METH) withdrawal during the first several weeks of abstinence. Several lines of evidence suggest the key role of brain-derived neruotrophic factor (BDNF) and its specific receptor, tropomyosin-related kinase (TrkB), signaling in the pathophysiology of depression. In this study, we examined whether BDNF-TrkB

signaling plays a role in the METH withdrawal symptoms (depression and behavioral sensitization). In the tail-suspension test, forced swimming test, 1% sucrose preference test, repeated administration of METH (3 mg/kg/day for 5 days) caused depression-like behaviors in mice, and depression-like behavior persisted more than 2-weeks after the final administration of METH. Western blot analysis showed that levels of BDNF and phosphorylated-TrkB in the nucleus accumbens (NAc) of METH treated mice were significantly higher than those of control mice although levels in the other regions, including prefrontal cortex, hippocampus, were not different. Furthermore, METH-induced depression and behavioral sensitization could be improved after subsequent repeated administration of TrkB antagonist ANA-12, but not TrkB agonist 7,8-dihydroxyflavone (7,8-DHF). Interestingly, METH-induced depression and behavioral sensitization could be improved after a single bilateral infusion of ANA-12 into NAc. These findings suggest that BDNF-TrkB signaling in the NAc plays a key role in the withdrawal symptoms in mice after repeated METH administration, and that TrkB antagonists would be potential therapeutic drugs for METH withdrawal symptoms in humans.

Disclosures: Q. Ren: None. **M. Ma:** None. **C. Yang:** None. **J. Zhang:** None. **W. Yao:** None. **K. Hashimoto:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.04/J34

Topic: C.17. Drugs of Abuse and Addiction

Support: NIHDA031259

Title: Acid-sensing ion channels control locomotor and rewarding effects of amphetamine

Authors: *X. CHU[1], Q. JIANG[2], C. HASSANZADEH[2], A. JIVAN[3];
[1]Basic Med. Sci., Univ. Missouri-Kansas City, Kansas City, MO; [3]Anesthesiol., [2]Univ. of Missouri-Kansas City, Kansas City, MO

Abstract: Drug addiction is a persistent mental illness and there is no effective therapy for patients. The precise mechanisms underlying addictive responses have not been completely deciphered. New evidence has been shown that ion channels in the brain reward circuits are believed to play a vital role in drug addiction. Acid-sensing ion channels (ASICs) are highly expressed in brain with ASIC1a and ASIC2 channels being the predominant subtypes. These channels are enriched at synaptic sites and are central for the regulation of normal synaptic transmission. Moreover, increasing evidence is linking ASICs to the pathogenesis of various neurological and neuropsychiatric disorders. We and others have shown that ASICs are involved in cocaine addiction. Here, we hypothesized that amphetamine, a psychostimuland similar to

cocaine, may also impact the function of ASICs. Following IACUC approval, adult wild-type (WT) C57BL/6J, ASIC1 and ASIC2 knock-out (KO) mice were placed in individual test chambers to allow accommodation to novel environment for 60 minutes. They then received a single intraperitoneal (i.p) injection of amphetamine at 3.0 mg/kg, and their locomotor activities were recorded for 150 minutes. The experiment was repeated daily for a total of 5 days. After a 2-week withdrawal period, the mice were brought back to the behavioral chamber followed by a final challenge i.p injection of amphetamine at 1.5 mg/kg. Locomotor activity to this challenge dose was measured for 150 min. Acute amphetamine injection induced a typical dose-dependent increase in locomotor activities in WT, ASIC1 and ASIC2 KO mice. However, the increase in locomotor activities were attenuated in ASIC1 and ASIC2 KO mice as compared to WT mice. Both WT, ASIC1 and ASIC2 KO mice showed sensitization to amphetamine. However, ASIC1 KO mice showed more, while ASIC2 KO mice showed less behavioral sensitization to amphetamine. Our data provides new understanding of the complex genetic and molecular mechanisms of ASICs in response to amphetamine exposure.

Disclosures: **X. Chu:** None. **Q. Jiang:** None. **C. Hassanzadeh:** None. **A. Jivan:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.05/J35

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA grant DA022473

NIH grant DA034140

NSF grant DGE-1144086

Title: Individual differences in methamphetamine self-administration model methamphetamine-addicted phenotype and are associated with cellular alterations in dentate gyrus of hippocampus

Authors: *M. H. GALINATO[1,2], M. FANNON[2], J. C. SOBIERAJ[2], A. GHOFRANIAN[2], A. I. NAVARRO[2], S. CHAING[2], S. S. SOMKUWAR[2], C. MANDYAM[2];
[1]UCSD, La Jolla, CA; [2]Committee on the Neurobio. of Addictive Disorders, The Scripps Res. Inst., La Jolla, CA

Abstract: The hippocampus is important for the relapse stage of addiction, and maladaptive patterns of methamphetamine intake result in altered levels of neurogenesis in the dentate gyrus during withdrawal. Individual differences in methamphetamine intake during extended-access self-administration were used to model the methamphetamine-addicted phenotype. These individual differences allowed us to test whether specific patterns of methamphetamine-intake

differentially altered proliferation and survival of neural stem cells, and granule cell neuron activation and structural plasticity in the dentate gyrus of the hippocampus. Male outbred Wistar rats were trained to self-administer methamphetamine 6 hr/day for 17 sessions (FR 1 schedule, 0.05mg/kg per IV infusion). Rats with higher levels of methamphetamine intake (high responders, n = 15) exhibited escalating patterns of drug intake across sessions, whereas the rats with lower levels of methamphetamine intake (low responders, n = 13) maintained a stable pattern of intake without escalation. High responders exhibited a vertical and rightward shift in the self-administration dose-response function and higher breakpoints on a progressive ratio schedule compared to low responders, indicating a preferred higher intake level and higher motivation. BrdU was injected during withdrawal and after three weeks of withdrawal, high responders demonstrated greater latency to extinguish drug-seeking behavior, greater drug-context-induced reinstatement and greater cue-induced reinstatement, indicating higher propensity for drug relapse compared with low responders. Brain tissue was processed for Golgi-Cox staining, and hippocampal sections were processed for Ki-67 (cell proliferation), BrdU (17-day-old surviving cells), AC3 (apoptosis), and cFos (neuronal activation) immunohistochemistry. Stereological analysis of cell numbers in the dentate gyrus resulted in an increase in expression of Ki-67, BrdU, and cFos in high responders, and an increase in AC3 in low responders compared to controls. 3D Sholl analysis of preexisting dentate gyrus granule cell neurons demonstrated no differences in dendritic complexity. The changes in behavior and associated cellular effects were not due to differential metabolism or bioavailability of methamphetamine since hippocampal methamphetamine levels were identical 45 min after a methamphetamine challenge in low and high responders. These findings suggest that methamphetamine addiction is related specifically to differential alterations in granule cell neurogenesis, and these alterations may be able to modulate methamphetamine-seeking behavior.

Disclosures: **M.H. Galinato:** None. **M. Fannon:** None. **J.C. Sobieraj:** None. **A. Ghofranian:** None. **A.I. Navarro:** None. **S. Chaing:** None. **S.S. Somkuwar:** None. **C. Mandyam:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.06/J36

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA033049

Title: Investigating the role of nucleus accumbens core astrocytes in reinstated methamphetamine seeking

Authors: M. D. SCOFIELD, K.-C. LEONG, C. GARCIA-KELLER, S. M. GHEE, C. A. THOMAS, P. W. KALIVAS, *C. M. REICHEL;
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Astroglial cells play an important role in the regulation of synaptic plasticity in the nucleus accumbens core (NAcore) through the regulated release and uptake of glutamate. Numerous studies show that exposure to various drugs of abuse causes reductions in the expression and or function of key components of the glial glutamate release (cystine-glutamate exchanger, system Xc-) and uptake (glial glutamate transporter, GLT-1) machinery. Importantly, these drug-induced neuroadaptations have been linked to relapse vulnerability. Consistent with other stimulants, methamphetamine (meth) self-administration followed by extinction training reduces extracellular glutamate in the NAcore leading to a potentiation of corticoaccumbal synapses, which underlies the enhanced glutamate release following exposure to drug-paired cues responsible for the initiation of drug seeking. We hypothesized that meth self-administration followed by extinction would also decrease expression and/or function of GLT-1. Strikingly, we did not observe any reduction of GLT-1 expression or glutamate uptake. Further, ceftriaxone, a β-lactam antibiotic, which has been shown to restore Xc- and GLT-1 expression, normalize glutamate uptake, and inhibit reinstated cocaine seeking, had no effect on cued meth seeking. However, restoration of NAcore glutamate tone via chronic N-acetylcysteine treatment during extinction inhibited cued-induced meth reinstatement. Additionally, activation of Gq-coupled designer receptors exclusively activated by designer drugs (DREADDs) in NAcore astrocytes prior to cue-induced reinstatement stimulated glial glutamate release and inhibited cued meth seeking, while having no impact on cued sucrose seeking. In summary, our data demonstrate that meth self-administration and extinction training did not impact accumbens glutamate uptake and that ceftriaxone treatment did not inhibit cued meth reinstatement. In contrast, restoration of extrasynaptic glutamate tone with N-acetylcysteine or activation of astroglial Gq-DREADD receptors reduced cue-induced meth seeking. Combined, our data indicate that similar to other dugs of abuse, potentiated synaptic glutamate release following exposure to meth-paired cues is responsible for the initiation of drug seeking and despite the lack of alterations in glutamate uptake, cued reinstatement of meth seeking is inhibited by the normalization of extrasynaptic glutamate tone provided by enhanced glial glutamate release.

Disclosures: M.D. Scofield: None. **K. Leong:** None. **C. Garcia-Keller:** None. **S.M. Ghee:** None. **C.A. Thomas:** None. **P.W. Kalivas:** None. **C.M. Reichel:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.07/J37

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA09397

Title: Neuron-specific modulation of dendritic spine dynamics in nucleus accumbens by amphetamine-paired contextual stimuli

Authors: *P. VEZINA[1], N. BUBULA[1], D. LI[1], V. BINDOKAS[2], B. F. SINGER[3];
[1]Dept Psychiatry and Behavioral Neurosci., [2]Dept Neurobiology, Pharmacology, and Physiol., The Univ. of Chicago, Chicago, IL; [3]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Repeated exposure to amphetamine leads to both associative conditioning and nonassociative sensitization. Here we assessed the contribution of immunohistochemically identified cFos+ and FosB+ medium spiny neurons (MSNs) in the nucleus accumbens (NAcc). Compared to saline exposed rats, animals exposed to amphetamine IP or in the ventral tegmental area (VTA) showed the expected sensitized locomotor response when challenged with amphetamine weeks later. Both exposure routes also increased FosB levels in medial aspects of the NAcc, suggesting a role for FosB+ MSNs in this site in the accrual and maintenance of sensitization. Further characterization of these FosB+ neurons with single-cell injections of the neuronal tracer DiI, however, revealed no differences between saline and amphetamine exposed rats in dendritic spine density or in spine tip diameter, indicating that these neurons do not undergo changes in dendritic spine morphology that accompany the expression of nonassociative sensitization. As drugs are necessarily administered in the presence of a large number of environmental stimuli, conditions favoring the formation of drug-stimulus associations, additional experiments determined how NAcc MSNs contribute to the expression of associative conditioning. In these experiments, a discriminative learning paradigm was used to expose rats to IP or VTA amphetamine either Paired or Unpaired with an open field. In addition, Paired rats received saline and Unpaired rats amphetamine in the home cage. Control rats received saline in both environments. As expected, Paired rats administered amphetamine IP showed a conditioned locomotor response and an increase in the number of cFos+ neurons in medial NAcc when subsequently challenged with saline in the open field. Paired rats previously exposed to VTA amphetamine showed no evidence for conditioned locomotion and no evidence for an increase in the number of cFos+ neurons. An increase in FosB+ neurons was observed in both Paired and Unpaired rats, again consistent with a role for these neurons in the accrual of sensitization but not in the expression of conditioning. Further characterization of the activated cFos+ MSNs revealed that IP amphetamine exposed Paired rats, compared to rats in the other groups, showed an increase in the density of dendritic spines and spine tips as well as in the frequency of medium-sized spine tip diameters. These findings suggest a role for cFos+ MSNs in the medial NAcc and specifically for rapid changes in the morphology of their dendritic spines in the expression of conditioned responses evoked by amphetamine-paired stimuli.

Disclosures: **P. Vezina:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH grant R01 DA09397. **N. Bubula:** None. **D. Li:** None. **V. Bindokas:** None. **B.F. Singer:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.08/J38

Topic: C.17. Drugs of Abuse and Addiction

Support: DA033049

Title: Therapeutic potential of perirhinal cortex DREADDs on methamphetamine-induced deficits in novelty recognition and relapse

Authors: *J. PETERS[1], M. D. SCOFIELD[2], S. M. GHEE[2], J. A. HEINSBROEK[2], C. M. REICHEL[3];
[1]Dept. of Neurosciences, [3]Neurosciences, [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: Background: Long-term methamphetamine (meth) abuse has been linked to certain cognitive impairments in humans. Similarly, in rats, chronic meth self-administration leads to deficits in novel object recognition memory, which relies upon the perirhinal cortex. One central question is: how do these cognitive deficits impact reinstated meth seeking? Here, our purpose was to determine whether synthetic activation of the perirhinal cortex with DREADDs could repair meth-induced novelty recognition deficits and to investigate the impact of these cognitive enhancing effects on relapse using a novel cue-choice reinstatement test. Methods: We used a viral-mediated gene transfer approach to infect perirhinal neurons with designer receptors exclusively activated by a designer drug (DREADDs of the hM3Dq variant) in order to activate neurons. All rats were infused with AAV2-hSyn-HA-hM3Dq-IRES-mCitrine vector (UNC Vector Core) bilaterally into the perirhinal cortex prior to meth self-administration, therefore allowing at least 4 weeks for DREADD expression to peak. Rats self-administered meth (0.02 mg/infusion, i.v.) along an FR1 schedule of reinforcement. After 7 daily 1-h sessions, rats were switched to 6-h daily access sessions for 14 days, and then underwent drug abstinence. Rats were tested for object recognition on abstinence day 7 and 8 or 14 and 15, and tested for novel cue-choice relapse on abstinence day 7 or 14. Neuronal activation was achieved by administering the designer drug clozapine-N-oxide (CNO, 10 mg/kg, i.p.); control rats received vehicle. Results: Chronic meth self-administration resulted in an escalation of meth intake over time and pronounced object recognition deficits. CNO administered immediately after object familiarization effectively restored object recognition in meth rats 90 min later. Twenty-four hours later, however, the therapeutic effects were no longer evident. In contrast, CNO had no impact on novel cue-choice reinstatement when administered 30 min prior to testing. Discussion: The data suggest that synthetic activation the perirhinal cortex is capable of restoring novelty recognition in chronic meth-exposed rats. However, these therapeutic effects did not extend to reduced relapse in a model incorporating choice between a novel cue and a meth cue. Further research is needed to determine whether CNO dose or pharmacokinetics can account for the lack of effect on relapse. Nonetheless, restoring cognitive function in meth addicts using a DREADD approach is a translationally attractive means to help sustain abstinence.

Disclosures: **J. Peters:** None. **M.D. Scofield:** None. **S.M. Ghee:** None. **J.A. Heinsbroek:** None. **C.M. Reichel:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.09/J39

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA036331

Phi Sigma, Beta Lambda Chpt. Weigel Grant

Title: Amphetamine affects reward-related behavior but not reward-evoked dopamine signals

Authors: ***D. R. SCHUWEILER**, J. M. ATHENS, J. M. THOMPSON, S. T. VAZHAYIL, P. A. GARRIS;
Sch. of Biol. Sci., Illinois State Univ., Normal, IL

Abstract: Dopamine (DA) transients in the nucleus accumbens (NAc) are brief increases in DA that are critical for reward-related learning. They are elicited by unpredicted rewards and learned cues that predict rewards but not predicted rewards. Thus, learning correlates with a "transfer" of NAc DA transients from rewards to their predictive cues. The DA transfer deficit theory of attention deficit hyperactivity disorder (ADHD) proposes that symptoms are caused by insufficient "transfer" and that psychostimulant medications compensate for this by enhancing cue-evoked DA transients. At therapeutic doses, amphetamine (AMPH) is the most efficacious treatment for ADHD; however, at larger doses AMPH is rewarding and can cause addiction. Rewarding doses of AMPH enhance DA transients, an effect thought to contribute to addiction via over-learning of drug-predictive cues. The effect of therapeutic doses of AMPH on DA transients has not been investigated; therefore, it is unclear if AMPH-induced enhancement of DA transients is specifically a mechanism of reward learning or also a therapeutic mechanism. We employed a Pavlovian autoshaping paradigm to examine AMPH dose effects in rats. This paradigm uses insertion of a lever and illumination of a light on one side of the chamber as a CS+; insertion of a lever and illumination of a light on the opposite side is used as a CS-. Some rats, goal-trackers, approach the food-trough when the cue is present while other rats, sign-trackers, approach the lever. Prior to the second of ten conditioning sessions, rats received an i.p. injection of saline or AMPH (0.25 or 1.36mg/kg d-amphetamine hemi-sulfate). Rats treated with the high dose increased sign-tracking and tended to require more sessions to learn to respond to the CS+ and not the CS-. We also combined fast-scan cyclic voltammetry with an unpredicted food reward paradigm to determine if alterations in reward-evoked DA transients might contribute to these behavioral outcomes. A carbon-fiber microelectrode was lowered into the

NAc until reward-evoked DA transients could be reliably recorded. 30 food pellets were delivered on a variable schedule both before and after administration of 0.25 or 1.36mg/kg AMPH. Additionally, electrically evoked phasic-like DA signals were recorded before and after the behavioral task. Only rats treated with the high dose exhibited increased electrically evoked signals. Surprisingly, the high dose had no significant effect on reward-evoked transients yet it tended to cause the rats to cease consuming the rewards. Taken together, these experiments suggest rewarding doses of AMPH alter learning by a mechanism that does not alter reward-evoked DA transients.

Disclosures: D.R. Schuweiler: None. **J.M. Athens:** None. **J.M. Thompson:** None. **S.T. Vazhayil:** None. **P.A. Garris:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.10/J40

Topic: C.17. Drugs of Abuse and Addiction

Support: USPHS Award DA026451

Title: Inhibition of AKT phosphorylation in the rat ventral tegmental area prevents intermittent social defeat stress-induced weight gain deficits and the expression of amphetamine cross-sensitization

Authors: *C. E. JOHNSTON[1], R. P. HAMMER, Jr[2,1], E. M. NIKULINA[2,1];
[1]Arizona State Univ. - Neurosci. Program, Tempe, AZ; [2]Basic Med. Sci., Univ. of Arizona Col. of Med., Phoenix, AZ

Abstract: Intermittent social defeat stress is an unpredictable stressor that produces cross-sensitization to amphetamine and a corresponding upregulation of mu-opioid receptors (MORs) in the ventral tegmental area (VTA). A population of VTA gamma-aminobutryic acid (GABA) neurons express MORs, which inhibit GABA release onto local dopamine neurons, and their expression is necessary for social stress-induced cross-sensitization to amphetamine. We previously showed that social stress increases the labeling of phosphorylated AKT (pAKT) preferentially in VTA GABA neurons, and that this effect is dependent on VTA MOR expression. We hypothesize that intra-VTA inhibition of pAKT during social defeat stress will prevent stress-induced amphetamine cross-sensitization and weight gain deficits. Adult male Sprague Dawley rats received bilateral cannulas directed at the VTA. Social defeat stress consisted of exposure to both threat of defeat and a brief physical defeat by an aggressive Long-Evans rat. Either saline vehicle or NVP-BEZ235 (10 μM in 1 μl per side), a dual inhibitor of phosphoinositide 3-kinase/mTOR signaling used to inhibit AKT phosphorylation, were infused 1

hr prior to each episode of social defeat stress or control handling, which occurred 4 times in 10 days. Intra-VTA inhibition of pAKT significantly blocked the development of long-term weight gain deficits observed in vehicle-treated stressed rats. Ten days after the last episode of defeat, all rats received an amphetamine challenge (1.0 mg/kg, i.p.). Intra-VTA inhibition of pAKT during stress did not alter the development of stress-induced amphetamine cross-sensitization, however when inhibitor was infused one week later prior to a second amphetamine challenge, it completely blocked the expression of cross-sensitization, as locomotor activity of stressed rats did not differ from handled animals after VTA pAKT inhibition. Taken together, these data implicate MOR-induced pAKT in the metabolic and behavioral effects of stress, suggesting that VTA pAKT may mediate stress-induced weight gain deficits and vulnerability to psychostimulants. Furthermore, that intra-VTA inhibition of pAKT blocked the expression, rather than the induction, of amphetamine cross-sensitization suggests that pAKT inhibition may provide a novel therapeutic approach for the treatment of substance abuse.

Disclosures: C.E. Johnston: None. **R.P. Hammer:** None. **E.M. Nikulina:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.11/J41

Topic: C.17. Drugs of Abuse and Addiction

Support: GAUK 88315

GACR 14-03708S

260168/SVV/205

PRVOUK P34

Title: Social play behavior in juvenile rats after neonatal exposure to methamphetamine

Authors: *M. SEVCIKOVA, A. HOLUBOVA, I. HREBICKOVA, R. SLAMBEROVA; Charles Univ. In Prague, Third Fac. of Med., Prague, Czech Republic

Abstract: Methamphetamine (MA) belongs to the most abused drugs. The popularity of this drug among women, and pregnant women as well, seems to be due its psychostimulant and anorectic effects. Suckling neonates can also be exposed to MA postnatally since MA is secreted in the mother´s breast milk. The neonatal period in rats corresponds in the development of the nervous system to the third trimester in humans. The aim of our study is to determine the effect of MA exposed during 11 days after birth to the social play. Social play is an integral part of the development of social behavior, marker of creating the hierarchy and cohesion of the group. Additionally, social play is known to be modulated by neurotransmitters system involved in

reward and motivation. Pups receiver MA during postnatal days (PD) 1-11 either directly (in dose of 5mg/kg s.c.) or indirectly via the breast milk of the mother (the same dosage). The control groups received saline in the same way. On the PD 28 and 29, the pups were individually habituated to the test cage for 10 min. Subsequently, the animals were socially isolated during the night before the testing day. On the PD 30, the pups were treated with MA (acute dose of 1mg/kg s.c.) or saline 45 minutes before testing. The test consisted of placing 2 similarly treated animals of the same sex into the test cage for 15 min. The frequency of social play behavior, which consists of pinning and pouncing, was significantly higher in the group of females receiving saline directly in comparison to MA group of the same type of application. Pups who received neonatal saline or MA directly played more compared to indirect application of saline or MA respectively. The acute application of MA eliminated social play in all the groups. Due to the psychostimulant effect of MA, animals of all the groups with acute application of MA displayed increased exploratory behavior such as rearing, sniffing, locomotion. Thus it seems that exposure of MA in neonatal period affects the social play behavior of the pups more when MA is administered via the maternal breast milk and the acute dose of MA suppresses the social play.

Disclosures: **M. Sevcikova:** None. **A. Holubova:** None. **I. Hrebickova:** None. **R. Slamberova:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.12/J42

Topic: C.17. Drugs of Abuse and Addiction

Support: 260045/SVV/2014

GACR 14-03708S

PRVOUK P34

Title: Sex dimorphic effect of acute methamphetamine treatment on behavior of adult rats exposed to the same drug throughout gestation and early neonatal period

Authors: *I. HREBÍCKOVÁ, M. SEVCIKOVA, R. SLAMBEROVA;
Charles University, Third Fac. of Med., Praha, Czech Republic

Abstract: Methamphetamine (MA) is a street drug causing potent psychomotor activation. Our previous studies demonstrated that effect of MA exposure on behavior of adult rats prenatally exposed to MA is sex-depended. The aim of the present study was to examine the effect of acute treatment of MA in adult rats that were exposed to MA during different phases of gestational and

early neonatal periods. Female rats were daily injected with MA (5 mg/kg) or saline during first (gestational day (GD) 1-11) or the second (GD 12-22) half of gestation period. These periods correspond to the human first and the second trimester of gravidity. The third trimester in humans corresponds to postnatal day (PD) 1-12 in rats. Therefore, MA was injected to rat mothers also in early lactation period. Their pups were exposed to the effect of drug indirectly via breast milk or directly with MA injection. Thus, four types of pre/postnatal administration periods were used in the present study. In adulthood, half of animals from each group were injected prior to the testing with MA (1mg/kg), second half received saline. Spontaneous locomotor activity and exploratory behavior were tested in Laboras apparatus (Metris B.V., Netherlands) for 1 hour. Our results demonstrated that acute administration of MA in all groups increased both, locomotion and exploration. Sex dimorphic effect of acute MA exposure was present in all groups. The females displayed more locomotion and more exploratory rearing than males in dependence of the time and kind of the application. Thus, our results indicate that adult MA injections affect behavior of adult rats in sex-, treatment- and time-exposure-specific manner.

Disclosures: I. Hrebícková: None. **M. Sevcikova:** None. **R. Slamberova:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.13/J43

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant R15DA035435

Title: Differential rearing alters amphetamine self-administration: role of mGluR2/3 activation

Authors: *D. ARNDT, E. GARCIA, G. ERICKSON, M. ULMER, M. CAIN;
Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Previous research has demonstrated that rats reared in an enriched environment self-administer less amphetamine at low unit doses than rats reared in isolation. We have recently demonstrated that differential rearing influences the function of post-synaptic metabotropic glutamate receptors (mGluRs) that contribute to the maintenance of glutamate homeostasis. The current study sought to determine if differential rearing also influences the function of presynaptic mGluRs critical for glutamate homeostasis, as research suggests that maintaining homeostatic glutamatergic function may be a protector against drug abuse. Male rats arrived to the lab at 21 days of age and were assigned to enriched (EC), isolated (IC), or standard (SC) conditions. EC rats were handled by experimenters and lived with cohorts and novel objects. IC rats lived with neither cohorts nor novel objects. SC rats lived in pairs in shoebox cages to

provide a lab standard for comparison. At 52 days of age, rats were trained to lever press for 20% sucrose on a fixed-ratio schedule (FR-1). Rats were then implanted with indwelling jugular catheters. Following surgery recovery, rats self-administered intravenous amphetamine (0.1 mg/kg/infusion) on a FR-1 schedule during 60 min daily sessions. After reaching stable responding, rats were injected with three doses (0, 0.3, and 1.0 mg/kg, i.p.) of the mGluR2/3 agonist, LY-379268 (LY), 30 min prior to self-administration sessions. Following FR-1 testing with all three doses, rats were tested with the same doses of LY on a progressive-ratio (PR) schedule following the same design as the FR-1 phase. Results revealed that LY generally decreased amphetamine self-administration under both FR-1 and PR schedules of reinforcement. Differential rearing influenced the attenuation of FR-1 self-administration. Specifically, EC rats given both 0.3 and 1.0 mg/kg LY earned significantly fewer amphetamine infusions than IC rats given the same doses. Furthermore, time course analyses revealed that LY led to a greater suppression of FR-1 and PR amphetamine self-administration in EC rats compared to IC rats. 1.0 mg/kg LY suppressed early-session FR-1 amphetamine self-administration in EC rats compared to IC rats, with no time course differences observed during vehicle FR-1 self-administration sessions between any of the environmental groups. These findings suggest the mGluR2/3 receptor may play a role in altering amphetamine self-administration among differentially reared rats, and differential rearing alters the function of pre- and post-synaptic mGluRs that maintain glutamate homeostasis.

Disclosures: D. Arndt: None. **E. Garcia:** None. **G. Erickson:** None. **M. Ulmer:** None. **M. Cain:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.14/J44

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH COBRE P20. GM104360

Title: Exposure to amphetamine during development changes drug response, histone methylation, and reduces dopamine uptake in Caenorhabditis elegans progeny

Authors: *T. J. MCCOWAN, B. D. SAFRATOWICH, L. CARVELLI;
Dept. of Basic Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: Amphetamine (AMPH) is a psychostimulant which is well known to increase dopamine (DA) in the synaptic cleft. *C. elegans* have been used in previous research to study AMPH because it possesses a well conserved dopaminergic system and display a well characterized behavioral response known as Swimming Induced Paralysis (SWIP), which results

from an increase in DA within the synaptic cleft. This behavior was used to investigate the young adult animals' response following embryonic AMPH exposure, as well as their progenies' response as young adult animals. Results show that animals who were exposed as embryos to AMPH (F0 AMPH) exhibited a higher SWIP response for the first 7 minutes when challenged with AMPH as young adults, with respect to control animals (F0 M9). Interestingly the progeny originating from animals previously exposed to AMPH (F1 AMPH) also exhibited a higher SWIP response during AMPH challenge with respect to the progeny of control animals (F1 M9). Western blots were performed to investigate histone methylation changes, as previous research has shown this epigenetic mechanism plays a role in addiction. A significant decrease was observed in histone H3 lysine 4 trimethylation (H3K4me3) in F1 AMPH with respect to F1 M9. Additionally a significant increase was observed in histone H3 lysine 9 dimethylation (H3K9me2) in F1 M9 with respect to both F1 AMPH and F0 M9. We are currently investigating whether AMPH changes these histone markers specifically in the dopaminergic neurons by performing staining in animals expressing GFP fused to the dopamine transporter (DAT). Previous data showed DAT plays a vital role in the SWIP response. To explore changes in the DAT as a potential mechanism for the changes we observed in SWIP, DA uptake assays were performed. Primary cell cultures were made from embryos of animals that were previously exposed to AMPH during development, as well as embryos of animals exposed to control solution. Our results show a significant decrease in DA uptake in cultures made from the embryos (F1) of animals previously exposed to AMPH during development, with respect to control cultures. As many of the components of the dopaminergic system as well as epigenetic mechanisms are highly conserved between *C. elegans* and mammals, these experiments could be critical for our understanding of how drugs of abuse affect future generations.

Disclosures: T.J. McCowan: None. **B.D. Safratowich:** None. **L. Carvelli:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.15/J45

Topic: C.17. Drugs of Abuse and Addiction

Title: Estimation of contribution of newly synthesized dopamine to amphetamine-induced increases in extracellular dopamine

Authors: *L. J. WALLACE, K. E. ROONEY, M. T. JANSON;
Ohio State Univ., Columbus, OH

Abstract: Amphetamine, a drug used to treat ADHD and narcolepsy and often used for non-medical reasons, increases extracellular dopamine. Data from both behavioral and micro-dialysis experiments suggest that newly synthesized rather than stored dopamine contributes a major

proportion of the increase in extracellular dopamine involved in the effects of amphetamine. The goal of this study is to determine the proportions of newly synthesized and stored dopamine contributing to amphetamine-stimulated increases in extracellular dopamine levels and to hypothesize a mechanism of secretion for newly synthesized dopamine. A representation of extracellular space associated with a single dopamine release site and 2,500 dopamine transporters was developed using MCell. Rates of dopamine secretion required for the model output to match published data for levels of extracellular dopamine after various doses of amphetamine were determined. These rates were separated into a stored (exocytotic) and non-stored (newly synthesized) component, with the stored component decreasing with increasing dose of amphetamine to account for drug induced decrease in firing rate of dopamine neurons. The non-stored component showed a biphasic dose response, with maximum at 1-2 mg/kg amphetamine, a pattern similar to that for published data on effects of amphetamine on rate of dopamine synthesis. A postulated mechanism for moving newly synthesized dopamine to extracellular space utilizes a membrane-associated complex of enzymes that convert tyrosine into DOPAC for export into extracellular space. Amphetamine inhibits monoamine oxidase in the complex, and the resulting accumulated dopamine is secreted in place of DOPAC. When this complex utilizes 20% of baseline and all of the amphetamine-stimulated increase in rate of dopamine synthesis, estimated dopamine secretion rates match the non-stored release rate determined in the model for doses of amphetamine up to 5 mg/kg. For higher doses of amphetamine, dopamine secretion from the proposed complex was not sufficient to provide high levels of extracellular dopamine needed to match published micro-dialysis data. Inclusion of decreased availability of active dopamine transporter for the highest dose of amphetamine does provide a model output that matches published data. The overall conclusion is that amphetamine-stimulated release of newly synthesized dopamine coupled with amphetamine competitive inhibition of dopamine transporter can account for measured levels of increased extracellular dopamine for most doses of amphetamine. At higher doses, however, the additional effect of decreased transporter availability is required.

Disclosures: **L.J. Wallace:** None. **K.E. Rooney:** None. **M.T. Janson:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.16/J46

Topic: C.17. Drugs of Abuse and Addiction

Support: PHS NIH AA020919 to SCS

PHS NIH DA035958 to SCS

NRF MEST 2012R1A6A3A03040362

Title: The effect of reactive oxygen species scavengers in methamphetamine-taking behaviors and dopamine release in the nucleus accumbens

Authors: *E. JANG[1], S. P. KIM[2], D. M. HEDGES[1], J. Y. LEE[2], T. EKINS[1], A. PEREZ[1], C. FREEMAN[1], A. LAMPRECHT[1], D. BRADSHAW[1], H. Y. KIM[2], C. H. YANG[2], S. C. STEFFENSEN[1];
[1]Psychology, Brigham Young Univ., Provo, UT; [2]Physiol., Daegu Haany Univ., Daegu, Korea, Republic of

Abstract: Methamphetamine (METH), a powerful and commonly used psychostimulant, has been shown to induce reactive oxygen species (ROS) formation, leading to oxidative stress, by dopamine (DA) auto-oxidation at DAergic terminals. Recently, we and others have implicated ROS in the development of behavioral sensitization following repeated contingent and non-contingent administration of psychostimulants such as cocaine. In this study, we evaluated the involvement of ROS in METH self-administration behavior and acute METH enhancement of DA release in the nucleus accumbens (NAc) using fast scan cyclic voltammetry (FSCV) *in vivo*. To evaluate the effect of ROS scavengers, rats received N-tert-butyl-α-phenylnitrone (PBN, a nonspecific ROS scavenger; 50 or 75 mg/kg, IP) or 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL, a SOD mimetic; 50 or 100 mg/kg, IP) 10 minutes prior to beginning of METH self-administration (0.05 mg/kg/infusion) and IV injection of acute METH (0.1 mg/kg). Systemic administration of PBN or TEMPOL significantly decreased METH self-administration without affecting food intake. Using 8-OHG immunohistochemistry, increased oxidative stress was found in the NAc of rats self-administering METH compared to sham controls. Acute administration of TEMPOL (100 mg/kg, IP) had no significant effect on the enhancement of DA release produced by acute METH. However, in preliminary studies, repeated administration of TEMPOL (25 mg/kg, 4 days, IP) decreased DA release produced by acute METH. Taken together, these findings indicate that enhancement of ROS production contributes to the reinforcing effect of METH.

Disclosures: **E. Jang:** None. **S.P. Kim:** None. **D.M. Hedges:** None. **J.Y. Lee:** None. **T. Ekins:** None. **A. Perez:** None. **C. Freeman:** None. **A. Lamprecht:** None. **D. Bradshaw:** None. **H.Y. Kim:** None. **C.H. Yang:** None. **S.C. Steffensen:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.17/J47

Topic: C.17. Drugs of Abuse and Addiction

Support: PHS NIH AA020919 to SCS

PHS NIH DA035958 to SCS

Title: Acute methamphetamine induces hydrogen peroxide formation in dopamine terminals of the nucleus accumbens

Authors: *D. HEDGES[1], E. JANG[2], A. W. PEREZ[2], N. D. SCHILATY[3], E. S. SCHRIEVER[1], N. FOLEY[1], B. R. BLUMELL[2], J. T. YORGASON[4], F. P. BELLINGER[5], J. D. UYS[6], S. C. STEFFENSEN[2];
[2]Psychology, [1]Brigham Young Univ., Provo, UT; [3]Ohio State Univ., Columbus, OH; [4]Oregon Hlth. & Sci. Univ., Portland, OR; [5]Univ. of Hawaii, Manoa, HI; [6]Med. Univ. of South Carolina, Charleston, SC

Abstract: Methamphetamine (METH) is a powerful psychostimulant known to both reverse the dopamine (DA) transporter (DAT) and inhibit the monoamine vesicular transporter (VMAT-2). Ultimately, METH facilitates DA release by causing reverse transport of DA through the DAT while simultaneously interfering with VMAT-2 pumping of DA into vesicles. Dopamine is subject to auto-oxidation and enzymatic degradation via MAO, resulting in formation of reactive oxygen species (ROS), specifically hydrogen peroxide (H2O2). The aim of this study was to evaluate the role of ROS in the effects of acute METH on phasic and basal DA release in the NAc. Utilizing a fluorescent dye sensitive to peroxide formation and fast-scan cyclic voltammetry (FSCV), superfusion of METH (1-100 uM) induced H2O2 production in the NAc. Using FSCV and the antioxidants glutathione (GSH) and 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), we evaluated the role of ROS in acute METH enhancement of both phasic (modeled by electrical stimulation) and basal DA release. Superfusion of METH (0.1-100 μM) dramatically increased phasic DA release in the NAc slice preparation (330% at 100 μM METH). However, the enhancement was transient, for within 15 min of continuous superfusion of METH DA release returned back to baseline, suggesting desensitization. This effect was difficult to wash out - over an hour later a similar challenge dose of METH was unable to raise the signal more than 5%. Glutathione (100 μM) was unable to attenuate the transient increase in phasic DA release, but was able to prevent METH's enhancement of basal DA release. Similarly, TEMPOL, a SOD mimetic, had no effect on phasic release, but attenuated basal release. Inhibiting DAT with GBR 12909 was unable to impact the effects of METH on phasic DA release, but inhibiting VMAT-2 with tetrabenazine reduced the effects of METH on phasic DA release. Since VMAT-2 appears to be playing a primary mechanistic role in basal release and a small role in phasic release, protein mechanistic studies are underway to show interactions between GSH and cysteine residues on VMAT-2.

Disclosures: D. Hedges: None. **E. Jang:** None. **A.W. Perez:** None. **N.D. Schilaty:** None. **E.S. Schriever:** None. **N. Foley:** None. **B.R. Blumell:** None. **J.T. Yorgason:** None. **F.P. Bellinger:** None. **J.D. Uys:** None. **S.C. Steffensen:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.18/J48

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R24 DA027318

P20-RR016467

G12MD007601

Title: Selenium deficiency alters dopamine transmission and response to methamphetamine in the mouse nucleus accumbens

Authors: D. J. TORRES[1], S. M. BARAYUGA[1], R. H. L. H. RUELI[1], D. M. HEDGES[2], N. D. SCHILATY[2], J. T. YORGASON[3], M. A. ANDRES[4], S. C. STEFFENSEN[2], *F. P. BELLINGER[1];
[1]CELL & MOLECULAR BIOL, JABSOM, Univ. Hawaii, Honolulu, HI; [2]Brigham Young Univ., Provo, UT; [3]Oregon Hlth. and Sci. Univ., Portland, OR; [4]PBRC, Univ. Hawaii, Honolulu, HI

Abstract: Selenium (Se) is an antioxidant trace element that is important for normal brain function. Se is incorporated into selenoproteins, a family of proteins with multiple functions that include protection from oxidative stress. Methamphetamine (METH) increases dopamine (DA) signaling by inhibiting DA reuptake, resulting in increased oxidative stress from oxidized DA, and eventual degeneration of DAergic terminals. Previous studies have indicated that Se protects against METH-mediated neurotoxicity, potentially through the antioxidant actions of the glutathione peroxidase (GPx) selenoenzymes. Conversely, Se-deficiency potentiates METH toxicity. To investigate the mechanisms of how dietary Se deficiency alters METH toxicity, we investigated DA concentrations and reuptake kinetics in the nucleus accumbens (NAc). We used fast-scan cyclic voltammetry (FSCV) to measure changes in extracellular DA in NAc brain slices following evoked release and changes following METH application. Se-deficiency impaired initial DA reuptake kinetics compared with slices from mice raised on a normal Se diet. This indicates reduced function of the dopamine active transporter (DAT). Se-deficiency did not alter the METH-induced increase in peak extracellular DA concentration. However, Se-deficiency did attenuate METH-induced impairments of DA reuptake kinetics. We additionally measured protein changes in the brains of Se-deficient mice compared to mice on a normal diet. Western blots demonstrated that Se-deficiency decreased levels of DAT and GPx compared to controls. These results suggest that Se-deficiency results in less availability of DA reuptake machinery, promoting DA toxicity and impairing responses to METH.

Disclosures: D.J. Torres: None. **S.M. Barayuga:** None. **R.H.L.H. Rueli:** None. **D.M. Hedges:** None. **N.D. Schilaty:** None. **J.T. Yorgason:** None. **M.A. Andres:** None. **S.C. Steffensen:** None. **F.P. Bellinger:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.19/K1

Topic: C.17. Drugs of Abuse and Addiction

Support: Mellon Foundation to ALR

Dean's Graduate Research Grant to BW

Dean's Undergraduate Research Grant to MMM

Title: Effect of preexposure on methylphenidate-induced taste avoidance and related BDNF/TrkB activity in the insular cortex of the rat

Authors: *B. WETZELL[1,2], M. M. MULLER[2], S. M. FLAX[2], H. E. KING[2], K. DECICCO-SKINNER[1], A. L. RILEY[2,1];
[1]Dept. of Biol., [2]Dept. of Psychology, American Univ., Washington, DC

Abstract: Exogenous brain-derived neurotrophic factor (BDNF) in the insular cortex (IC) is known to influence conditioned taste avoidance (CTA) learning, but little is known of its endogenous role in the phenomenon. A history with many abusable compounds attenuates their ability to induce CTA (known as a preexposure effect), thus providing a possible platform from which to examine the endogenous role of IC BDNF in CTA. In the present study, this role was examined by assessing the effect of preexposure to methylphenidate (MPH) on MPH-induced CTA, followed by an analysis of changes in expression between preexposure groups of BDNF and its primary receptor, the tropomyosin-related kinase receptor type B (TrkB) in the IC, central nucleus of the amygdala (CeA) and the nucleus accumbens (NAc). Specifically, following preexposure to 18 mg/kg MPH, CTAs induced by 0, 10, 18 and 32 mg/kg MPH were assessed in adult male Sprague Dawley rats (n = 64). In separate groups of rats (n = 31), differences in BDNF and TrkB were assessed using Western blots following similar preexposure and conditioning procedures. In line with previous research with psychostimulants, preexposure to MPH significantly blunted MPH-CTA compared to preexposure to vehicle. Although there were no significant effects of MPH on BDNF activity following CTA conditioning, animals preexposed to MPH exhibited decreased BDNF/TrkB activity in the CeA and enhanced activity in the IC and NAc. Thus, preexposure to MPH attenuates its aversive effects on subsequent presentations, and BDNF's endogenous impact on CTA learning may be dependent upon its temporal relation to other CTA-related intracellular cascades.

Disclosures: B. Wetzell: None. **M.M. Muller:** None. **S.M. Flax:** None. **H.E. King:** None. **K. Decicco-Skinner:** None. **A.L. Riley:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.20/K2

Topic: C.17. Drugs of Abuse and Addiction

Title: Homer2 regulates sensitivity to methamphetamine reward

Authors: *C. BROWN[1], S. G. QUADIR[2], D. M. FLAHERTY[2], K. K. SZUMLINSKI[2]; [1]UCSB Psychological and Brain Sci., Santa Barbara, CA; [2]UCSB, Santa Barbara, CA

Abstract: Homer2 is a post-synaptic scaffolding protein involved in regulating glutamate receptor function, which has been highly implicated in drug-induced neural plasticity. Withdrawal from repeated methamphetamine injections increases Homer2 protein expression within the shell, but not the core, subregion of the nucleus accumbens, although vulnerability to high MA reward is associated with elevated Homer2 expression within both accumbens subregions. Herein, we employed 2 strategies to test the hypothesis that Homer2 regulates the motivational valence of methamphetamine. The first study employed constitutive Homer2 gene knock-out (KO) mice and demonstrated that KO mice expressed conditioned place-aversion to an environment paired repeated with low-dose methamphetamine (1 mg/kg). The second study employed an shRNA knockdown approach to selectively lower Homer2 expression within either the shell or core subregion of C57BL/6J mice. Homer2 knock-down within the shell, but not the core, attenuated the expression of a methamphetamine-conditioned place-preference and this effect was observed when animals were tested in under methamphetamine-free and -primed states. Together, these results indicate that Homer2, particularly within the nucleus accumbens shell, contributes to the rewarding properties of methamphetamine.

Disclosures: C. Brown: None. **S.G. Quadir:** None. **D.M. Flaherty:** None. **K.K. Szumlinski:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.21/K3

Topic: C.17. Drugs of Abuse and Addiction

Title: vmPFC infusion of mGlu2/3 receptor agonist during protracted withdrawal does not prevent incubation of cocaine-seeking

Authors: *C. B. SHIN, M. A. RUPPERT-MAJER, M. M. SERCHIA, J. R. SHAHIN, T. E. KIPPIN, K. K. SZUMLINSKI;
Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Relapse to drug-taking is a reoccurring phenomenon impairing addiction recovery that can be triggered by the elicitation of intense drug craving upon re-exposure to drug-paired cues. Cue-elicited drug-craving increases in a time-dependent manner during drug abstinence - a phenomenon termed “incubation of craving”. The neural substrates of this phenomenon are not fully understood but are thought to involve glutamate mechanisms in the ventromedial prefrontal cortex (vmPFC) as the capacity of cocaine-paired cues to increase glutamate release within vmPFC incubates during protracted withdrawal in concert with behavior. We hypothesize that incubated cue-elicited glutamate release within vmPFC might drive incubated behavior. To test this hypothesis, male Sprague-Dawley rats were trained to lever-press for cocaine (0.25 mg/infusion; 6 h/day) for 10 consecutive days. At 30 days withdrawal, animals were infused intra-vmPFC (0.5 µl /side) with either vehicle or 50µM of the mGlu2/3 autoreceptor agonist APDC and underwent a 30-min test for cue-elicited cocaine-seeking. A control group was infused with vehicle at 3 days withdrawal to provide a base-line response. Vehicle-infused animals exhibited a time-dependent intensification of cue-reinforced responding, indicating incubation. However, the magnitude of this incubation was not influenced by 50µM APDC. As mGlu2/3 receptor down-regulation is reported within the medial PFC of rats withdrawn from non-contingent cocaine treatment, current studies seek to characterize mGlu2/3 expression within the vmPFC following a history of excessive cocaine intake to better understand the molecular mechanism(s) underpinning the cue hyper-reactivity of vmPFC glutamate that presumably drives incubated cocaine-seeking.

Disclosures: C.B. Shin: None. **M.A. Ruppert-Majer:** None. **M.M. Serchia:** None. **J.R. Shahin:** None. **T.E. Kippin:** None. **K.K. Szumlinski:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.22/K4

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA grant AA016650 to KKS

Title: Withdrawal-dependent incubation of anxiogenesis and dipsomania following a history of binge alcohol drinking during adolescence

Authors: *K. M. LEE, M. A. COEHLO, N. R. SOLTON, T. E. KIPPIN, K. K. SZUMLINSKI; Univ. of California At Santa Barbara, Santa Barbara, CA

Abstract: Binge alcohol drinking is the most prevalent form of alcohol abuse exhibited by adolescents and young adults, yet little is known regarding the short- or longer-term impact of binge drinking during this sensitive period of neurodevelopment upon brain and behavior. Herein, we characterized the short- and longer-term effects of a relatively short (14-day) period of binge alcohol drinking during adolescence upon emotionality, in comparison to adults with equivalent binge-drinking experience. Male C57BL/6J mice binge-drank alcohol under 4-bottle-choice procedures (5, 10, 20, 40% v/v) for 2 hrs/day, starting 3hrs into the dark cycle. Adolescent bingers commenced drinking on PND 28, while adults commenced on PND 56. A subset of adolescents were tested for behavior in early withdrawal (PND42), while all the other animals underwent behavioral testing on PND 70, followed by an additional 5 days of drinking. There was no age difference in the average alcohol intake over the initial 14-day period, although adolescents preferred 40% alcohol, while adults preferred 20% alcohol. When tested in early withdrawal, adult drinkers exhibited hyper-anxiety in the defensive marble burying test and, corroborating published data from humans and rat, "hang-over"-related anxiety was absent in adolescent bingers. However, when assayed for behavior in protracted withdrawal (PND70), the hyper-emotionality and subsequent alcohol intake exhibited by adolescent bingers was greater than age-matched adults. These data provide evidence that both anxiety and dipsomania "incubate" with the passage of time during alcohol withdrawal in individuals with an early life history of binge drinking and further the argument that binge drinking during adolescence perturbs the developmental trajectory of neurocircuits underpinning emotional control and control over alcohol intake.

Disclosures: K.M. Lee: None. **M.A. Coehlo:** None. **N.R. Solton:** None. **T.E. Kippin:** None. **K.K. Szumlinski:** None.

Poster

316. Amphetamine and Related drugs: Neural Mechanisms of Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 316.23/K5

Topic: E.03. Behavioral Neuroendocrinology

Title: Rottlerin and psychostimulant-induced conditioned place preference.

Authors: *T. LIAO[1], L. YU[2];
[1]Inst. of Behavioral Medicine, Natl. Cheng K, Kaohsiung, Taiwan; [2]Natl. Cheng Kung University, Col. of Medicine, Inst. of Behavioral Med., TAINAN, Taiwan

Abstract: Brain-derived neurotrophic factor (BDNF) has been known to modulate cocaine conditionings. Systemic rottlerin (RO) administration can increase hippocampal BDNF levels in a long-lasting manner. Accordingly, we hypothesized that systemic pretreatment with RO may affect psychostimulant-induced conditioned place preference (CPP). To test this hypothesis, 8-week-old male C57BL/6 mice were used to receive an intraperitoneal RO injection (5 mg/kg) approximately 15 hour prior to the first bout of the saline-environment conditioning. Our CPP training protocol consisted of 3-day conditionings [saline-environment conditionings were in the morning, while psychostimulant-environment conditionings were in the afternoon at an 8-hour inter-conditioning interval]. We found that such RO treatment decreased methamphetamine (MA) (1 mg/kg/conditioning)- and cocaine (10 mg/kg/conditioning)-induced CPP. Likewise, pretreatment with 7,8-dihydroxyflavone (10 mg/kg, i.p.), a selective BDNF TrkB receptor agonist, before each saline-, cocaine-conditioning was effective in decreasing the cocaine(10 mg/kg/conditioning)-induced CPP. In an attempt to assess the modulating effects of RO on the maintenance of psychostimulant-induced CPP, a single RO injection was given approximately 24 hours before the 3-day forced extinction protocol [two saline-environment conditionings for each day]. We found that RO (5 mg/kg) did not affect the forced extinction protocol-produced decreases in the MA- or cocaine-induced CPP magnitude. Nevertheless, RO seemed to effectively abolish the MA-primed reinstatement of the extinguished MA-induced CPP. These results, taken together, suggest that systemic rottlerin administration may be beneficial in facilitating the erasure of the methamphetamine-supported memory.

Disclosures: **T. Liao:** None. **L. Yu:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.01/K6

Topic: C.17. Drugs of Abuse and Addiction

Support: DA12136

MD007599

Title: Sex differences in novel object recognition after a binge methamphetamine treatment

Authors: ***A. KLAMBATSEN**[1,2], S. NYGARD[3], V. QUINONES-JENAB[1], S. JENAB[1]; [1]Psychology, Hunter Col., New York, NY; [2]The Grad. Center, CUNY, New York, NY; [3]Washington Univ., St. Louis, MO

Abstract: Methamphetamine is a well-established neurotoxin that selectively damages dopaminergic cells, causing cell death and destruction of dopamine terminals. Behavioral evidence also indicates an effect on dopamine-dependent activities, as exemplified by the novel

object recognition task. However, few studies have examined the effects of methamphetamine on females. This study sought to test the sex differences in memory impairment using the novel-object recognition task. A four-dose methamphetamine “binge” dosage paradigm (4 x 5mg/kg, 2 hours apart) was used to compare memory performance between males, intact females, and ovariectomized females. Drug-treated males and ovariectomized females, but not intact females, exhibited memory impairments compared to saline animals. No differences in total exploration time were observed based on sex or drug condition. Methamphetamine toxicity-induced behavioral deficits therefore appear to differentially impact individuals based on sex.

Disclosures: A. Klambatsen: None. **S. Nygard:** None. **V. Quinones-Jenab:** None. **S. Jenab:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.02/K7

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant HL126559

NIH Grant MH098891

NIH Grant DA027569

NIH Grant MH072567

NIH Grant MH63022

Title: Methamphetamine-induced aberrant neurogenesis: protection by exercise

Authors: *M. TOBOREK, H. LEVINE, M. PARK;
Biochem. and Mol. Biol., Univ. of Miami Sch. of Med., Miami, FL

Abstract: While no effective therapy is available for the treatment of methamphetamine (METH) induced neurotoxicity, behavioral interventions, including aerobic exercise, are being used to improve depressive symptoms and substance abuse outcomes. The present study focuses on the effect of exercise on METH-induced neurotoxicity in the hippocampal dentate gyrus (DG) in the context of the blood-brain barrier (BBB) pathology. METH or saline (vehicle) was administered three times per day for 5 days with an escalating dose regimen at 3 h intervals. One set of mice was sacrificed 1 day post last injection of METH and the remaining mice were divided into two major groups: a) the exercise group and b) the sedentary group. After chronic METH administration, the expression of tight junction (TJ) proteins was decreased in the hippocampus. Importantly, BBB permeability was significantly increased and remained elevated

even 20 days after the withdrawal of METH. Moreover, neuronal differentiation was significantly decreased in METH-exposed hippocampal DG, suggesting impaired neurogenesis. Most importantly, voluntary exercise protected against this effect, enhanced the protein expression of occludin, and inhibited induction of inflammatory cytokines. These results suggest that exercise can attenuate METH-induced neurotoxicity by protecting against the BBB disruption and related microenvironmental changes in the hippocampus.

Disclosures: **M. Toborek:** None. **H. Levine:** None. **M. Park:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.03/K8

Topic: C.17. Drugs of Abuse and Addiction

Support: Regis University URSC Funding to MM, ZV, KV, and AFG

Title: The effect of exercise on the neurochemical consequences of methamphetamine abuse

Authors: ***M. MURRAY**, Z. VLASTOS, K. VARLEY, A. N. FRICKS-GLEASON;
Regis Univ., Denver, CO

Abstract: Abuse of methamphetamine (METH) in the Unites Stated has increased significantly in the past 15 years, and use is now endemic in the Western states. Colorado currently ranks 7th in the nation for total number of METH users over the age of 25. Overall, the economic cost of drug abuse is high. METH abuse alone costs the U.S. $23.4 billion annually due to crime, lost workplace productivity, foster care, and other social problems stemming from abuse. In addition to the known health risks associated with psychostimulant abuse, METH use carries the additional danger of permanent brain injury. One well-known animal model of METH use utilizes binge METH administration, where repeated doses of METH are given to rats in a single day. This dosing regimen has been shown to cause long-lasting damage to dopaminergic nerve terminals and serotonergic nerve terminals similar to that seen in human METH abusers. In humans, it has been suggested that METH-induced monoaminergic damage may lead to the development of Parkinson's disease. Exercise is a non-pharmacological treatment being explored for use in treating Parkinson's disease and this work has recently been extended to the study of METH-induced monoaminergic neurotoxicity. It has been shown that when rats exercised for 3 weeks before and 3 weeks after a binge treatment of METH, this exercise significantly attenuated METH-induced decreases in striatal dopamine. Interestingly, if the exercise regimen was limited to only 3 weeks before a binge treatment of METH, it did not protect against striatal dopamine damage. This suggests that pre-METH exercise does not help with prevention of neurotoxicity, but perhaps post-METH exercise aids in recovery. This study specifically tested

the effects of 3 weeks of exercise after a METH binge on the recovery of dopaminergic nerve terminals in the striatum and serotonergic nerve terminals in the prefrontal cortex. By examining the timing of the intervention, the results presented here provide important information about the therapeutic relevance of exercise as a potential treatment for METH-induced neurotoxicity and the concomitant cognitive deficits.

Disclosures: **M. Murray:** None. **Z. Vlastos:** None. **K. Varley:** None. **A.N. Fricks-Gleason:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.04/K9

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant P30 GM103328

Title: Consequences of self-administered methamphetamine throughout pregnancy on rat dams and their offspring

Authors: *D. RÜEDI-BETTSCHEN, S. CHAWLA, C. S. WASHINGTON, D. M. PLATT; Psychiatry and Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Methamphetamine (METH) abuse in women of childbearing age and those who are pregnant is an urgent health concern. While the harmful effects of METH are well described for adults, there is only limited knowledge of the effects of METH use during pregnancy on the developing child. In the present study, we investigated how daily METH self-administration throughout pregnancy affected rat dams, as well as how the resulting *in utero* METH exposure affected offspring development through weaning. Yoked saline control dams and their offspring served as controls. Female rats (n=6/group) were trained to self-administer METH (0.08 mg/kg/infusion) under a fixed-ratio schedule such that every injection of METH by a dam also resulted in a saline injection to her yoked control dam. When stable levels of self-administration were reached, all females were mated. Daily self-administration sessions continued until litters were born. General health and weight was assessed daily in dams and pups. In addition, pups were evaluated for achievement of age-appropriate developmental milestones. METH females self-administered 2-3 mg/kg/day prior to mating and throughout gestation. This level of METH self-administration had no effect on dam health, dam behavior or pregnancy outcome. Weight gain throughout pregnancy (and after) did not differ between METH dams and saline controls. All females produced viable litters, and litter size, composition and pup weight at birth did not differ between saline and METH dams. Similarly, maternal pup-directed behavior was not affected by prior METH self-administration experience. In contrast to the dams, pups were

negatively affected by *in utero* METH exposure. Compared to saline-exposed pups, METH exposed pups were delayed in reaching developmental milestones, including righting reflex, eye opening, incisor eruption and negative geotaxis. These results demonstrate the feasibility of using self-administration of METH by dams as a means to expose developing pups to the drug. More importantly, the results indicate that *in utero* exposure to low-to-moderate METH doses can profoundly affect offspring development, suggesting that even moderate or recreational METH use during pregnancy adversely affects offspring development.

Disclosures: D. Rüedi-Bettschen: None. **S. Chawla:** None. **C.S. Washington:** None. **D.M. Platt:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.05/K10

Topic: C.17. Drugs of Abuse and Addiction

Support: VA Grant 1I01RX000458

Title: Differential dopaminergic toxicity of bath salt intermediates in mice: Implications for the mechanism of methamphetamine toxicity

Authors: *J. H. ANNEKEN[1,3], M. ANGOA-PEREZ[1,3], G. SATI[2], D. CRICH[2], D. M. KUHN[1,3]; [2]Dept. of Chem., [1]Wayne State Univ., Detroit, MI; [3]John D. Dingell VAMC, Detroit, MI

Abstract: “Bath salts” are a relatively novel class of stimulants, which consist of cathinone and its derivatives (e.g., cathinone, mephedrone, and methylone). These exhibit a close structural similarity to corresponding amphetamine class compounds (amphetamine, methamphetamine, and MDMA, respectively). Despite this similarity, the bath salts have been shown to lack the reduction in dopaminergic markers elicited in mice by methamphetamine and other neurotoxic amphetamines. The difference in toxicity between these closely related compounds presents a unique opportunity to better understand the mechanisms by which methamphetamine elicits its toxic effects in the brain. The bath salt mephedrone differs from methamphetamine by both a β-keto group and a 4-methyl group on the phenyl ring of its structure. In order to assess which of these additions may be responsible for the observed loss of dopaminergic toxicity, the intermediate compounds, methcathinone (MeCa) and 4-methylmethamphetamine (4MM), were administered at a range of doses to mice every 2 h in the standard binge regimen for a total of 4 injections, and dopaminergic markers were assessed in the striatum 48 h following treatment. In comparison to methamphetamine, 4MM lacked any striatal dopaminergic toxicity with the sole exception of a slight but significant decrease in dopamine at 40 mg/kg, which was the highest dose mice were able to tolerate. 4MM also exhibited significant, dose-dependent

hyperlocomotion, increased stereotyped behavior, and hyperthermia, similar to methamphetamine. As has been observed with other bath salt compounds, 4MM significantly enhanced the dopaminergic toxicity of a mildly toxic dose of methamphetamine (2.5 mg/kg) at 20 and 40 mg/kg, but not 10 mg/kg. In contrast with 4MM, MeCa exhibited significant reductions in striatal dopamine, DAT, and TH at the higher doses tested (20-80 mg/kg), as well as a significant increase in the inflammatory marker GFAP. MeCa induced hyperthermia and hyperlocomotion at lower doses, but at 80 mg/kg, induced significant hypothermia. The higher doses tested also elicited increased stereotyped behavior. These data support the view that while both the β-keto and 4-methyl substitutions lead to a lower neurotoxic potency when compared to methamphetamine, it is the 4-methyl ring-substitution on mephedrone that primarily prevents dopaminergic toxicity. Future experiments will investigate the mechanism by which this substitution leads to a loss of toxicity.

Disclosures: **J.H. Anneken:** None. **M. Angoa-Perez:** None. **G. Sati:** None. **D. Crich:** None. **D.M. Kuhn:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.06/K11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA07606

Title: Neurotoxic consequences of serial exposure to alcohol and methamphetamine

Authors: *A. L. BLAKER, B. K. YAMAMOTO;
Neurosciences, Univ. of Toledo, Toledo, OH

Abstract: Methamphetamine (Meth) and alcohol have an established comorbid relationship in drug abuse. Despite the widespread co-abuse of these drugs, little is known about the consequences arising from their serial exposure. Glutamate signaling is altered by alcohol and Meth administered independently, and glutamate-induced excitotoxicity mediates Meth neurotoxicity. Therefore, we tested the hypothesis that the serial exposure to alcohol and Meth will alter glutamatergic transmission and result in greater brain monoamine depletions than either drug alone. Male Sprague Dawley rats were exposed to a one week chronic binge of ethanol (6g/kg/day) via oral gavage followed by one day of binge Meth administration (10mg/kg x 4 injections). One week after treatment, the monoamine neurotransmitter content of the prefrontal cortex, striatum, and hippocampus was measured. Ethanol alone did not result in any monoamine depletions, while rats treated with Meth alone showed a 50% depletion of dopamine in striatum, as well as a 25% depletion of serotonin in striatum, hippocampus, and prefrontal cortex.

Importantly, ethanol significantly enhanced the neurotoxicity observed after Meth alone in that rats treated with both drugs showed 90% dopamine depletions within the striatum and 75% serotonin depletions in the striatum, hippocampus and prefrontal cortex. Other markers of monoamine terminals, such as dopamine transporter (DAT), serotonin transporter (SERT) and tyrosine hydroxylase (TH) immunoreactivities in the striatum were also measured one week after treatment. Western blot analysis showed that DAT, SERT and TH immunoreactivities were decreased 80% in the striatum of ethanol + Meth rats, compared to 40% decreases in rats exposed to Meth alone and no changes after ethanol alone. This enhanced effect after serial exposure to ethanol and Meth suggests a potential synergism between the drugs. To determine a role for glutamate in mediating monoamine depletions, the excitatory amino acid transporter 1 (EAAT1) was examined. EAAT1 immunoreactivity in the prefrontal cortex was decreased by 24% at one day after a week of ethanol alone. These results suggest a potential role for diminished glutamate uptake in mediating a vulnerability to excitotoxicity produced by ethanol, which could potentially be enhanced by Meth. Future studies will examine the role of excitotoxicity in mediating neurotoxicity after alcohol and Meth.

Disclosures: A.L. Blaker: None. **B.K. Yamamoto:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.07/K12

Topic: C.17. Drugs of Abuse and Addiction

Support: This research was supported by a grant (14182MFDS979) from Ministry of Food and Drug Safety in 2014, Republic of Korea

DK Dang, TV Tran, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea

Title: P47 phox contributes to induce methamphetamine dopaminergic neurotoxicity

Authors: D.-K. DANG[1], Y. NAM[1], T.-V. TRAN[1], C.-G. JANG[2], E.-J. SHIN[1], *H.-C. KIM[1]; [1]Col. of Pharmacy, Kangwon Natl. Univ., Chunchon, Korea, Republic of; [2]Sch. of Pharmacy, Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Accumulating evidences suggest that oxidative stress mediates MA-induced dopaminergic neurotoxicity. Since recent reports emphasized emerging role for NADPH oxidases as a source of ROS, we investigated whether involvement of NADPH oxidase in MA-induced proapoptosis (i.e., TUNEL-positive cells), microglial activation, and dopaminergic impairment in the striatum of the mice. As the p47 phox subunit of NADPH oxidase acts as a connector between the components of the membrane and the cytoplasm, we performed

intracerebroventricular infusion of p47 phox antisense oligonucleotides (p47 phox ASO) before a single injection of MA (35 mg/kg, i.p). Treatment with MA resulted in a significant increase in TUNEL-positive cells in the striatum of Taconic ICR mice. Application of p47phox ASO significantly attenuated the increase in TUNEL-positive populations induced by MA. Furthermore, p47 phox ASO significantly protected reactive microgliosis (as labeled by Iba-1) and reductions in tyrosine hydroxylase-positive immunoreactivity and dopamine level in the striatum of Taconic ICR mice. Consistently, reactive microgliosis and dopaminergic loss induced by MA were less pronounced in p47 phox knockout mice than in wild type mice. Our results suggest that p47 phox mediates acute dopaminergic neurotoxicity induced by MA.

Disclosures: D. Dang: None. **Y. Nam:** None. **T. Tran:** None. **C. Jang:** None. **E. Shin:** None. **H. Kim:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.08/K13

Topic: C.17. Drugs of Abuse and Addiction

Support: FDA protocol E7259.01

FDA protocol E7519.01

Title: Comparison of C57 B6N versus C57 B6 (NCTR strain) mice response to methamphetamine-induced seizures gives insights into the role of temperature, BBB breakdown and seizures on neurotoxicity

Authors: *J. F. BOWYER[1], K. M. TRANTER[1], S. SARKARA[1], J. RAYMICK[1], L. C. SCHMUED[1], J. P. HANIG[2];
[1]Neurotoxicology, NCTR/FDA, Jefferson, AR; [2]CDER/ FDA, Silver Spring, MD

Abstract: C57 B6 mice were used to further determine whether the blood-brain barrier (BBB) break down that occurs after a 40 mg/ kg methamphetamine (METH) s.c. is necessary for generating the status epilepticus and prominent neurodegeneration seen in limbic regions. Charles River (CR) C57 B6 mice were selected in lieu of the NCTR sub strain of C57 B6 (no longer available). Unexpectedly, differences in the seizuregenic and neurotoxic effects of METH in the C57 B6N mice from CR compared to the NCTR mice gave new insights into processes underlying these two events. BBB breakdown was evaluated in mice histologically at time points ranging from 15 min to up to 3 d after a single dose of D-METH. Fluoro-Gold (F-G, 30 mg/ kg i.p.) was given to some mice prior to sacrifice to help identify BBB breakdown. IgG immunoreactivity in brain was also used to detect BBB damage. In the C57 B6N, some mice had signs of continuous (10 to 30 min) seizure activity (SA) ranging in intensity from tremors to

Racine scale level 3 seizures but had body temperatures (BT) of only 39.6°C and 40.3°C. When BTs were <40.3°C there was no evidence of BBB damage at time points between 15 and 60 min after METH and no neurodegeneration at 1 or 3 d post METH. In mice with BTs of ≥ 40.9°C, IgG and F-G localization (intense spots < 1 mm in dia.) were seen in the anterior bed nucleus of the stria terminalis (BSTMA), anterior thalamus and hypothalamus, and amygdala nuclei of the accessory olfactory system (PLCo, PMCo) at 15 to 40 min after the first appearance of Racine scale 5 seizures and/ or status epilepticus. In this group of mice, with more intense SA, signs of neurodegeneration occurred within 4 h in the same areas where IgG appeared 15 to 40 min post METH. However, the only intense areas of neurodegeneration seen at 3 d in the C57 B6N were in the PLCo, PMCo and BSTMA. The maximal intensity and area of IgG immunoreactivity and neurodegeneration in the limbic regions of the C57 B6N was much less than that previously observed in the NCTR C57 B6J mice. Conclusions: in C57 B6N mice 1) METH can induce repetitive (≤30 min) mild to moderate SA in the absence of severe hyperthermia (<40.0°C); 2) However, under these conditions BBB breakdown does not occur and little or no forebrain neuronal cell death is detected; 3) when body temperatures are reach ≈ 41°C an early BBB breakdown in the BSTM, anterior hypothalamus and thalamus and amygdala nuclei occur which in turn likely exacerbates seizure activity and increases neurodegeneration. Additional studies are necessary in Jackson Laboratories original C57 B6J to determine whether the intensity and regional distribution of BBB breakdown 15 to 60 min after METH explain their greater sensitivity to METH neurotoxicity.

Disclosures: **J.F. Bowyer:** None. **K.M. Tranter:** None. **S. Sarkara:** None. **J. Raymick:** None. **L.C. Schmued:** None. **J.P. Hanig:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.09/K14

Topic: C.17. Drugs of Abuse and Addiction

Support: CONACyT grants No. 138663

CONACyT grants No. 129303

Title: Amphetamine addiction produces neuronal death in dorsal hippocampus and an increase of metabolites related with oxidative stress

Authors: ***L. ARROYO GARCÍA, SR**[1], H. TENDILLA BELTRAN[1], R. A. VÁZQUEZ-ROQUE[1], A. D. DIAZ FONSECA[1], E. BRAMBILA COLOMBRES[1], E. MONJARAZ GUZMÁN[1], F. DE LA CRUZ LÓPEZ[2], G. FLORES ALVARES[1];

[1]Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Inst. Politecnico Nacional, México Df, Mexico

Abstract: There are different addiction theories that try to explain the human behavior in this alteration, one of these is the sensitization theory of addiction which propose that drugs produce alterations in the mesocortical limbic system that attribute in the reward-stimuli, become hypersensitive the circuits involve in drug-seeking and cause the pathological incentive motivation. Amphetamine (AMPH) has been commonly used to produce this theory, which is known like amphetamine-sensitization, this drug induces an enhanced in dopaminergic tone in the mesocortical limbic system. In another hand now around 1.2% of the world population is AMPH addicted, this represent a social problem inducing a pathological state of neuronal mechanism of learning and memory. In our previous results using the same protocol, we found a decreasing for the long-term memory by the novel object recognition (NOR), and changes in some oxidative stress metabolites like oxide nitric (NO) and Zinc (Zn), which was correlated with the changes in neural morphology to the dorsal hippocampus (DH), since this region has an important role in memory and learning behaviors, required for the development in the human life. Now we know DH changes can be relevant, highlights the fact to understand the processes that affect this region, like changes in metabolites related with oxidative stress, in consequence with a decreasing in neural density and neuronal connectivity. The aim of the present study was measured the changes in neuronal density by stereological methods, neuronal connectivity, presence of astrocytes and quantify malonilaldehyde (MDA), metallothioneins (MTs) and caspase 3 (CASP3). Our results suggest that amphetamine sensitization in rats shown an altered relation between in MDA and MTs levels, which we correlate with an increase in GFAP and CASP3 with a decrease in SYP and neuronal density in hippocampal region. In conclusion, this study demonstrates that the damage caused by AMPH addiction produce neuronal death in hippocampus regions that is involved in behaviors required for the daily life. (Supported by: CONACyT grants No. 138663 and 129303 to G Flores).

Disclosures: **L. Arroyo García:** None. **H. Tendilla Beltran:** None. **R.A. Vázquez-Roque:** None. **A.D. Diaz Fonseca:** None. **E. Brambila Colombres:** None. **E. Monjaraz Guzmán:** None. **F. De la Cruz López:** None. **G. Flores Alvares:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.10/K15

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA07427

Title: MDMA reduces markers for GABAergic neurons in the hippocampus and increases seizure susceptibility: Role of glutamate mediated excitotoxicity

Authors: *C. L. HUFF[1], J. P. HERMAN[1], B. K. YAMAMOTO[2], G. A. GUDELSKY[1]; [1]Univ. of Cincinnati, Cincinnati, OH; [2]Univ. of Toledo Sch. of Med., Toledo, OH

Abstract: MDMA is a unique psychostimulant that continues to be a popular drug of abuse. It is well documented that MDMA produces persistent reductions in markers of 5-HT axon terminals in rodents, as well as humans. To date there has been little recognition of potential MDMA neurotoxicity to neuronal populations beyond 5-HT axon terminals in brain regions, such as the hippocampus, in which damage may account for the neurologic/cognitive effects associated with repeated exposure to MDMA. In the present study, we examined the hypothesis that MDMA produces glutamate-dependent damage to GABAergic neurons, as assessed from GAD67-positive neurons in the hippocampus, which results in an increase in seizure susceptibility. Repeated exposure to MDMA (3x10mg/kg, ip) resulted in a marked reduction in the number of GAD67 positive cells in the dentate gyrus, as well as in the CA1 and CA3 regions. Repeated administration of MDMA also resulted in an increased susceptibility to kainic acid-induced seizures that persisted for at least 30 days following MDMA treatment. Kainic acid (9 mg/kg, sc) produced seizures in approximately 20% of control animals, whereas approximately 85% of MDMA-treated animals exhibited kainic acid-induced seizures. The MDMA-induced increase in seizure susceptibility was not evident in rats treated with either MK-801 (a NMDA antagonist) or ceftriaxone (an inducer of GLT-1). In further support for a role of glutamate-mediated excitotoxicity in the MDMA-induced loss of hippocampal GABA neurons and increase in seizure vulnerability, is the finding that repeated treatment with MDMA results in an increased extracellular concentration of glutamate in the hippocampus that is also prevented in rats treated previously with ceftriaxone.

Disclosures: C.L. Huff: None. **J.P. Herman:** None. **B.K. Yamamoto:** None. **G.A. Gudelsky:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.11/K16

Topic: C.17. Drugs of Abuse and Addiction

Support: DA07606

DA07427

Title: MDMA decreases paired-pulse depression and afterdischarge threshold in the dentate gyrus: Roles of 5HT2a and EP1 receptor activation

Authors: *S. A. COLLINS[1], G. GUDELSKY[2], B. YAMAMOTO[1];
[1]Dept. of Neurosciences, Univ. of Toledo, Toledo, OH; [2]James Winkle Col. of Pharm., Univ. of Cincinnati, Cincinnati, OH

Abstract: MDMA is a widely abused psychostimulant which causes the release of serotonin through its actions on the serotonin transporter. Recent findings in our lab demonstrated that MDMA causes an increase in extracellular glutamate concentrations within the dentate gyrus, which was dependent upon local activation of 5HT2A receptors. These increases in glutamate were also dependent upon local activation of EP1 receptors, suggesting a role for PGE2 signaling in mediating these glutamate increases. Here we report that local administration of MDMA (100 μM) causes a significant increase in PGE2 concentrations within the dentate gyrus (246%, p=.016) of rats. PGE2 increases were inhibited when MDL100907, a 5HT2a receptor antagonist, was administered with MDMA. Previously, we reported a significant decrease in parvalbumin (PV) interneurons in the dentate gyrus following MDMA exposure, which could be prevented by inhibition of either 5HT2a or EP1 receptors during MDMA exposure. Given the previously demonstrated decreases in PV interneurons following MDMA exposure, we investigated whether MDMA alters inhibition within the dentate gyrus. Perforant path evoked field potentials in the dentate gyrus of MDMA treated rats exhibited a reduced paired-pulse depression at 40, 50 and 65 ms interstimulus intervals (41.5, 47.2, 36.4%, p=.001, .003, .006 respectively), 10 days following MDMA exposure (7.5 mg/kg x 4, ip). Decreases in paired-pulse depression were prevented by treatment during MDMA exposure with either MDL100907 or SC-51089, an inhibitor of EP1 receptors. Further experiments revealed a decrease in the stimulus amplitude needed to drive perforant path induced afterdischarges in the dentate gyrus of MDMA treated rats (19.4%, p=.001). Reductions in afterdischarge threshold were prevented when rats were treated during MDMA exposure with either SC-51089 or MDL100907. These findings suggest that MDMA causes a decrease in inhibition within the dentate gyrus, which may disrupt the excitatory/inhibitory balance. Furthermore, these findings suggest that MDMA-induced reductions in PV interneurons are responsible for decreases in inhibition. Further studies are needed to characterize the changes in GABAergic inhibition within the hippocampus of MDMA treated animals and whether these changes mediate known deficits in hippocampal function caused by MDMA.

Disclosures: S.A. Collins: None. **G. Gudelsky:** None. **B. Yamamoto:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.12/K17

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA-IRP, NIH

Title: Clinically-relevant pharmacological strategies that reverse MDMA-induced brain hyperthermia potentiated by social interaction

Authors: *E. A. KIYATKIN, S. REN, K. T. WAKABAYASHI, M. H. BAUMANN, Y. SHAHAM;
Behavioral Neurosci Br., NIDA-IRP, NIH, DHHS, Baltimore, MD

Abstract: MDMA-induced hyperthermia is highly variable, unpredictable, and greatly potentiated by the social and environmental conditions of recreational drug use. Current strategies to treat pathological MDMA-induced hyperthermia in humans are palliative and marginally effective, and there are no specific pharmacological treatments to counteract this potentially life-threatening condition. Here, we injected rats with a moderate non-toxic dose of MDMA under conditions modeling recreational drug use (i.e., social interaction) that led to the dramatic enhancement of MDMA-induced brain hyperthermia. We tested the effectiveness of mixed adrenoceptor blockers carvedilol and labetalol, and the atypical antipsychotic clozapine in reversing MDMA-induced hyperthermia. To mimic the clinical situation of drug intoxication, we injected the treatment drugs after MDMA had already caused robust hyperthermia (>2.5°C). Brain temperature was our primary focus, but we also simultaneously recorded temperatures from the deep temporal muscle and skin, allowing us to determine the basic physiological mechanisms of the treatment drug action. Carvedilol induced skin vasodilation and was modestly effective in attenuating MDMA-induced brain and body hyperthermia, whereas labetalol was ineffective. In contrast, clozapine induced a marked and immediate reversal of MDMA-induced hyperthermia via inhibition of brain metabolic activation and blockade of centrally-mediated vasoconstriction. Our findings suggest that clozapine, and related centrally acting drugs, can be highly effective for reversing MDMA-induced brain and body hyperthermia in emergency clinical situations, with possible life-saving results. Supported by NIDA-IRP.

Disclosures: E.A. Kiyatkin: None. **S. Ren:** None. **K.T. Wakabayashi:** None. **M.H. Baumann:** None. **Y. Shaham:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.13/K18

Topic: C.17. Drugs of Abuse and Addiction

Support: DA036012

DA019447

DA013367

Title: Sex-differences in rodent methamphetamine self-administration

Authors: *A. JOHANSEN, A. E. FLECKENSTEIN, A. E. FLECKENSTEIN, L. M. MCFADDEN;
Univ. of Utah, Salt Lake City, UT

Abstract: Methamphetamine (METH) is a widely abused psychostimulant that can cause persistent alterations in brain neurochemistry. METH is abused in both females and males. Research has shown that women exhibit higher co-morbid neuropsychiatric disorders and prefer METH to other drugs compared to their male counterparts. However, little research has been conducted to understand these sex-differences. The purpose of the present study was to investigate possible sex-differences in the behavioral and neurochemical effects of METH self-administration. Male and female rats were subjected to 7 days of self-administration (8 hours/day) of either METH or saline and were sacrificed one hour after the last self-administration session. METH-induced changes in hippocampal brain derived neurotrophic factor (BDNF) and expression of the striatal dopamine transporter (DAT) were assessed. Similar METH self-administration occurred between the sexes; however, METH-induced hyperthermia was significantly greater in females. METH self-administration decreased striatal DAT immunoreactivity in both females and males. METH-induced increases in hippocampal BDNF immunoreactivity occurred in males but not in females. In conclusion, there are similar drug-taking behaviors between the sexes, but sex-differences exist in the neurochemical consequences of METH self-administration. These findings may have clinical implications to sex-differences observed in human METH users.

Disclosures: A. Johansen: None. **A.E. Fleckenstein:** None. **A.E. Fleckenstein:** None. **L.M. McFadden:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.14/K19

Topic: C.17. Drugs of Abuse and Addiction

Support: 1R01HD070888-01A1

Title: Sex differences in the developmental and behavioral effects ofchronic oral methylphenidate in rats

Authors: *M. MICHAELOS[1], L. S. ROBISON[1], J. GANDHI[1], E. MIAO[1], C.-Y. LAM[1], A. MAUCERI[1], M. VITALE[1], J. LEE[1], S. PAENG[1], D. E. KOMATSU[1], M. HADJIARGYROU[2],

P. K. THANOS[1];
[1]Stony Brook Univ., Stony Brook, NY; [2]NYIT, New York City, NY

Abstract: Background: Methylphenidate (MP) is a widely prescribed psychostimulant for the treatment of Attention Deficit Hyperactivity Disorder (ADHD), that has received attention for its abuse potential and side effects which may persist after treatment cessation. Previously, we developed a dual dosage oral MP drinking paradigm for rats that mimics the pharmacokinetic profile of treated patients (Thanos et al. 2015). This study aims to follow up on previous data on the skeletal effects of chronic MP (Komatsu et al 2012) and to determine the developmental and behavioral effects of chronic oral MP treatment in both male and female rats. Methods: Male and female Sprague Dawley rats were assigned to 1 of 3 treatment groups (n=12/group) at 4 weeks of age: control (water), low dose (LD) MP, and high dose (HD) MP. Using the dual bottle-drinking paradigm, rats would drink 4 mg/kg MP (LD) or 30 mg/kg MP (HD) for one hour, and 10 mg/kg (LD) or 60 mg/kg MP (HD) for seven hours each day. Throughout the 3 months of treatment, rats in each group were monitored for body weight, food and fluid intake, as well as tested for open field activity behavior, circadian sleep-wake activity, novel object recognition, and social interaction behavior. Results: Chronic MP treated rats exhibited reduced fluid intake during treatment weeks. MP dose-dependently decreased body weight accrual for both genders, while not influencing overall food intake. MP dose-dependently increased locomotor activity in both sexes, and to a greater extent in females. MP-induced hyperactivity was observed during the dark cycle of their circadian dark cycle without affecting light cycle activity. MP had an anxiolytic effect that was similar in males and females. Chronic MP treatment had no effect on novel object recognition or social behavior for either sex. Conclusions: Chronic oral MP treatment at clinically-relevant doses was found to have significant effects on development and behavior, including body weight, locomotor activity, and anxiety. Particularly marked sex differences were most apparent for locomotor activity, with females being more sensitive to the hyper-activating effects of the drug. The lack of effects seen in novel object recognition and social behavior may be the result of neuroadaptations that occurred under chronic MP treatment. These findings suggest that chronic MP exposure beginning in adolescence can have significant behavioral effects that are both drug and sex-dependent. These findings raise concerns regarding the reversibility of these effects post-discontinuation of treatment, as well as how MP may influence the dopamine mesolimbic reward system and subsequent vulnerability for addiction.

Disclosures: **M. Michaelos:** None. **L.S. Robison:** None. **J. Gandhi:** None. **E. Miao:** None. **C. Lam:** None. **A. Mauceri:** None. **M. Vitale:** None. **J. Lee:** None. **S. Paeng:** None. **D.E. Komatsu:** None. **M. Hadjiargyrou:** None. **P.K. Thanos:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.15/K20

Topic: C.17. Drugs of Abuse and Addiction

Support: NSFC 81271472

Title: Segregated effects of 4-methylethcathinone on CPP, locomotor sensitization and anxiety-like behavior

Authors: P. XU[1], Y. QIU[2], P. XU[1], Y. LIU[1], *H. SHEN[2];
[1]Drug Intelligence and Forensic Center, Ministry of Publ. Security, Beijing, China; [2]Natl. Inst. On Drug Dependence, Peking Univ., Beijing, China

Abstract: Background: A second generation of synthetic cathinones 4-Methylethcathinone (4-MEC) has ranked among the most popular "legal highs" recently. Although 4-MEC appears to have similar neurochemical actions like cocaine mixing with MDMA *in vitro*, its behavioral profiles in human and experimental animals were little known Methods: This study investigated the addictive potential and psychomotor stimulation of 4-MEC (1-30 mg/kg) by measuring the conditioned place preference (CPP) and locomotor activity, while methamphetamine (METH, 1-3 mg/kg) was used as positive control. Because synthetic cathinones often cause adverse psychiatric sequelae, we then assayed the acute and chronic 4-MEC-induced anxiety/restlessness-like behavior using the elevated plus maze (EPM). Results: We found CPP was induced by 4-MEC at 10mg/kg after trainings and was able to reinstate after two weeks withdrawal. Although the last dose elicited an acute increase on locomotor activity, 4-MEC failed to induce locomotor sensitization with 3, 10 or 30 mg/kg. Rats treated by chronic 4-MEC with 30 mg/kg daily spent remarkably less proportion of open arm time compared to chronic saline group, while METH-treated rats showed augmentation in exploratory behavior. Interestingly, two weeks withdrawal after chronic 4-MEC or METH, all rats emerges to show more exploratory behavior rather than anxiety-like behavior. Conclusions: Taken together, these results revealed that 4-MEC-associated reward is segregate from its effects on locomotor stimulation. And, the effects of 4-MEC on addictive potential and restless behavior will be enduring even after long-term withdrawal.

Disclosures: **P. Xu:** None. **Y. Qiu:** None. **P. Xu:** None. **Y. Liu:** None. **H. Shen:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.16/K21

Topic: C.17. Drugs of Abuse and Addiction

Support: W.M. Keck Foundation Medical Research Award

Title: Cocaine-paired cues contribute to the acquisition, but not escalation, of intravenous cocaine self-administration in rats

Authors: ***P. A. VIEIRA**[1,2], L. BUBALO[1], K. L. PLOENSE[1], J. BAGLEY[1], C. SHIN[1], K. NOVICK[1], R. BOZADJIAN[1], T. E. KIPPIN[1,2];
[1]Psychological & Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA; [2]Inst. for Collaborative Biotechnologies, Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Cocaine addiction is a major health concern which is associated with excessive cocaine use. A leading animal model of excessive drug use is the expression of escalated cocaine intake during extended (6 h or greater) daily sessions of self-administration. Emerging evidence indicates that drug-related contextual cues are important in the maintenance of escalated cocaine intake as well as reinstatement of drug-seeking behavior. Similarly, discreet cocaine-paired cues have been extensively examined in the reinstatement of drug-seeking behavior as well as acquisition of cocaine taking but the role of these cues in the development and maintenance of escalated cocaine self-administration has not been examined. Here, we examined the impact of cocaine-paired cues on the acquisition and escalation of cocaine self-administration in a rat model. Rats were implanted with a permanent jugular catheter and then allowed to lever press to self-administer cocaine (0.25 mg/0.1 ml infusion, FR1, 20s time-out) during 5 daily acquisition (1-h) sessions followed by 15 extended-access (6 h) sessions. For one group, each cocaine infusion was paired with a 20s presentation of cue light above the active lever and, for a second group, no cue light was presented. Rats that received presentation of the cue light exhibited higher intake levels throughout the acquisition phase than those that did not ($P < 0.05$), but no differences were detected between groups during the escalation phase (cue rats exhibited 25.5±16.5% and no cue rats exhibited 30.7±8.3% escalation of intake across extended access sessions; $P > 0.05$). Further, following development of escalated cocaine intake, reversal of cue conditions failed to impact cocaine self-administration in either group. The present findings indicate that cocaine-associated discrete cues facilitate initial drug taking under limited access conditions but do not appear to contribute to the escalation of cocaine intake which contrasts with the reported role of contextual cues in the control of escalated cocaine intake.

Disclosures: **P.A. Vieira:** None. **L. Bubalo:** None. **K.L. Ploense:** None. **J. Bagley:** None. **C. Shin:** None. **K. Novick:** None. **R. Bozadjian:** None. **T.E. Kippin:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.17/K22

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA-027115

NIH Grant DA-027525

Title: Extended-access to cocaine has a distinct behavioral and molecular profile from yoked- and limited-access to cocaine

Authors: *K. PLOENSE[1], L. BUBALO[2], A. CARR[3], T. KIPPIN[2];
[1]Univ. of California Santa Barbara, Goleta, CA; [2]Univ. of California Santa Barbara, Santa Barbara, CA; [3]Neurosci., Wake Forest Univ., Salem, NC

Abstract: Cocaine addiction is a chronic disorder that involves escalation of intake over time. Approximately 10% of the US population has used cocaine by the age of 18, yet, only about 1% of US citizens meet the criteria for cocaine addiction. Both humans and rodents will self-administer escalating doses of cocaine when the drug is readily available. However, when cocaine access is restricted, escalation of intake is not apparent in rodents. Here, we investigated escalation of cocaine intake and active lever responding in rats as a function of control over intake. Rats were implanted with a permanent jugular catheter and then allowed to lever press to self-administer (FR1, 20s time-out with a 20s light cue paired with each infusion) saline vehicle (0.1 ml/infusion) cocaine (0.25 mg/infusion) under 4 conditions: limited-access (1 h/ day) to saline, limited-access to cocaine, extended-access (6 h/day) to cocaine condition, and limited-access + yoked-access (1h/day + 5 h/day, respectively) to cocaine. Based on the first 10 min and first hour of daily access, we observed rapid escalation of cocaine intake in both the extended-access and limited-access + yoked conditions (ps < 0.05). We also observed a delayed escalation of cocaine intake in the limited-access condition within the first 10 min of self-administration ($p < 0.05$), but not within the first 1 h of self-administration. Interestingly, there was an immediate escalation of active lever responding in the limited + yoked-access condition during the first 10 minutes of daily self-administration which preceded the escalation of responding observed in the extended-access condition. A similar pattern of escalation for active lever responding was observed during the first 1 h of daily self-administration for all cocaine conditions. However, relative to the other cocaine conditions, the limited-access + yoked group exhibited markedly less efficient self-administration (i.e. more non-reinforced relative to reinforced lever responses) during both the first 10 min and 1 h of daily self-administration (ps<0.05). . Additionally, post-mortem quantification of *homer2* (a gene implicated in cocaine cued learning) mRNA expression within the dmPFC indicated elevation only in the extended-access conditions ($p<0.05$). Together, these findings indicate that either contingent or non-contingent "excessive" cocaine exposure supports escalation but has distinct effects on the temporal patterning of operant responsiveness as well as molecular correlates of escalation.

Disclosures: K. Ploense: None. **L. Bubalo:** None. **A. Carr:** None. **T. Kippin:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.18/K23

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grants K25-DA021200

NIH Grants R21-DA032228

NIH Grants R01-DA029718

NIH Grants R01-NS084817

Title: Chronic cocaine disrupts angiogenesis and cerebral blood flow in the mouse brain

Authors: *K. PARK[1,2], J. YOU[1], J. CHOI[1], N. D. VOLKOW[2], C. DU[1], Y. PAN[1];
[1]Stony Brook Univ., Stony Brook, NY; [2]Natl. Inst. on Drug Abuse, NIH, Bethesda, MD

Abstract: Cocaine-induced stroke is among the most serious medical complications associated with its abuse. However, the extent to which chronic cocaine-induced reductions in cerebral blood flow (CBF) and micro-ischemia affects cerebral blood vessels has not been investigated, in part, because of the lack of tools with high spatiotemporal resolution and sensitivity that can simultaneously measure cerebral blood flow velocity (CBFv) quantitatively and morphology with single vascular resolution over large fields of view. Recently, we developed novel optical imaging techniques that permitted the quantitative CBF imaging of cerebrovascular networks along with the imaging of cortical vascular angiography simultaneously. Our study showed that CBF was decreased in response to cocaine and the micro-ischemia was observed in the cortex of the animal after repeated cocaine administration. Here we extend the study to investigate the effects of chronic cocaine on CBF and in the morphology of cerebral blood vessels. To monitor the neurovascular changes from chronic cocaine exposure in mice, a cranial window was implanted on the cortex of each individual mouse. Two groups of animals were used; a control group (saline, ~0.1cc/10g/day, i.p. 35 days) and a cocaine group (30mg/kg/day, i.p. 35 days). Each animal was periodically scanned to image CBFv and angiography of the cortical vascular network simultaneously till the end of treatment. Our repeated imaging of the cortex through the implanted cranial window showed vasoconstrictive effects of cocaine on the brain vessels with chronic cocaine treatment. Vessel constriction induced decreases in CBFv as compared to their baseline (prior to cocaine treatment). The diameters of vessels were decreased ~ 25-35% after 35 days of cocaine exposure compared to baseline, whereas CBFv in these vessels was decreased ~ 40-50%. Interestedly, angiogenesis surrounding the constricted vessels was observed by 12-14 days of cocaine treatment. Blood flow gradually developed in these new growing vessels and 7-8 days later blood flow to the local area had increased 20-30% and was maximal at the end of 35 days of our experiment. These results indicate that the angiogenesis is a response to repair the local micro-ischemia of brain induced by cocaine. Although the angiogenesis intends to improve blood perfusion into the ischemic brain area, the limited CBFv within these vessels makes them difficult to fully compensate for cocaine-induced cerebrovascular dysfunction.

Disclosures: **K. Park:** None. **J. You:** None. **J. Choi:** None. **N.D. Volkow:** None. **C. Du:** None. **Y. Pan:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.19/K24

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA015351

Title: Amphetamine and morphine may produce aspects of acute withdrawal by initially affecting a common pathway

Authors: *W. WHITE, R. WARD, I. WHITE;
Psychology, Morehead State Univ., Morehead, KY

Abstract: When separate groups of rats are administered 2.0 mg/kg amphetamine or 5.0 mg/kg morphine near light onset of a 12-12 hour light-dark cycle, activity is reduced 18 to 24 hours later. This longer-term hypoactivity may be an indicator of an acute withdrawal. If a dopamine D1 receptor antagonist is given shortly after amphetamine or morphine, the reduction in activity appears to be blocked. The similar time courses with which amphetamine and morphine produce hypoactivity, and the similar effects of D1 antagonist on the occurrence of the hypoactivity, suggest that the drugs produce it by initially activating a common circuit. This study sought additional evidence that amphetamine and morphine produce longer-term hypoactivity via a common pathway. Though all rats show longer-term hypoactivity following amphetamine or morphine, the magnitude differs across subjects. If amphetamine and morphine produce longer-term hypoactivity by initially activating a common circuit, then the magnitude of hypoactivity following amphetamine and morphine might be expected to be correlated, the hypothesis assessed in the present study. Adult male Wistar rats were individually housed in open field arenas (43 cm X 43 cm X 30 cm high), equipped with grids of infrared emitters and detectors. Beam interrupts were used to monitor distance moved per unit time, our measure of activity. The animals were in a 12-12 hour light-dark cycle and had free access to water and food (Purina rodent chow). After animals had habituated to the arenas and entrained to the light-dark cycle, they were tested at intervals of five days. On the first day of a test (Day 1), near light onset, animals received a saline administration. Two days later (Day 3), near light onset, they received 2.0 mg/kg amphetamine or 5.0 mg/kg morphine. Station maintenance occurred at the time of a treatment. Activity was monitored for 24 hours following Day 1 and Day 3 treatments. Activity following drugs was compared to activity following saline. During the first six tests, amphetamine was administered, and during the final tests, morphine was administered. The magnitude of hypoactivity that different animals showed following amphetamine and morphine appeared to be qualitatively similar.

Disclosures: W. White: None. **R. Ward:** None. **I. White:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.20/K25

Topic: C.17. Drugs of Abuse and Addiction

Support: NWO: Rubicon fellowship 446-14-015

NIMH: 1R01MH090134

NIDA: 5R21DA034954-03

NIDA: 1K01DA037452-01A1

NIDA: 1F32DA033088

Title: Disruption of the hubs of the connectome in cocaine addiction. A multimodal approach

Authors: *A. ZILVERSTAND[1], R. O'HALLORAN[1,2], P. KUNDU[1,2], M. A. PARVAZ[1], S. J. MOELLER[1], G. GAN[1], F. D'OLEIRE UQUILLAS[1], N. ALIA-KLEIN[1], R. Z. GOLDSTEIN[1]; [1]Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Radiology, Mount Sinai, New York, NY

Abstract: Brain networks or 'connectomes' are organized around highly connected processing 'hubs', which are particularly valuable for efficient information processing. Disruptions of these crucial information processing hubs have been linked to impairments in multiple neuropsychiatric disorders, including dementia, schizophrenia, and post-traumatic stress disorder. A recent meta-analytic study has demonstrated that structural microlesions in hub regions predict disruptions of these regions in resting-state functional connectivity. Using a novel multimodal neuroimaging approach, we aim to investigate both if the functional resting-state connectome in cocaine addiction shows disruptions specifically in brain processing hubs, and if these disruptions are predicted by structural microlesions. We acquired structural, diffusion-weighted and functional resting-state data in cocaine users (n=30) and healthy controls (n=33), matched on race, gender and intelligence. Per individual, a whole-brain functional connectome was derived from the resting-state data following standard procedures: parcellating the data according to an anatomical template, calculating functional connectivity between each pair of regions, and thresholding each connectome to contain only the strongest connections. Diffusion-weighted data were parcellated using the same template, and the number of fiber tracts connecting each pair of regions was computed. Brain connectivity (or degree) was defined as the number of connections between each region with all other brain regions. Structural data was processed using the same template, computing changes in gray matter volume by voxel-based morphometry. Functional and structural connectomes of cocaine addicts were contrasted with controls', controlling for age (p<0.05, uncorrected). Ongoing work is using logistic regression to

determine associations between structural and functional connectome abnormalities. Cocaine users showed disruptions of resting-state connectivity in processing hubs, such as the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (dlPFC), hippocampus, putamen and caudate that have previously been implicated across brain disorders. The ACC, dlPFC, and hippocampus also showed reduction in structural brain connectivity. We expect these disruptions to correlate with abnormalities in gray matter volume. Results support functional and structural abnormalities in brain network hubs within the cognitive control network in cocaine addiction. Evidence of their correlation could advance a multimodal systems-level account of the neural circuitry involved in compulsive behavior.

Disclosures: **A. Zilverstand:** None. **R. O'Halloran:** None. **P. Kundu:** None. **M.A. Parvaz:** None. **S.J. Moeller:** None. **G. Gan:** None. **F. d'Oleire Uquillas:** None. **N. Alia-Klein:** None. **R.Z. Goldstein:** None.

Poster

317. Amphetamines and Cocaine

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 317.21/K26

Topic: C.17. Drugs of Abuse and Addiction

Support: Internal funding, TMII, Mount Sinai

NIDA T32-DA007135-31 (KB)

NIDA R21DA034954 (RZG)

Title: Characterizing atherosclerosis in asymptomatic cocaine addicted individuals

Authors: *K. BACHI[1], V. MANI[2], R. Z. GOLDSTEIN[1], Z. A. FAYAD[2], N. ALIA-KLEIN[1]; [1]Psychiatry and Neurosci., [2]Translational Mol. Imaging Inst. (TMII), Icahn Sch. of Med. MSSM, New York, NY

Abstract: Cocaine use is involved in 40% of emergency department visits, where positive toxicology for illicit drugs have been associated with stroke, coronary artery disease and myocardial infarction, resulting in severe impairments or sudden mortality, even in absence of prior vascular disease symptoms. Indeed, cocaine, a powerful vasoconstrictor and a sodium channel blocker, decreases basal anti-inflammatory markers (Interleukin 10) and increases pro-inflammatory cytokines (Tumor necrosis factor alpha), contributing to progressive vascular inflammation (atherosclerosis). The carotid arteries supply blood to brain regions that are implicated in the higher-order cognitive impairments documented in individuals with cocaine use disorder (iCUD). Hence, structural and/or functional damage in the carotid arteries may impact cognitive and behavioral functioning even before substantial arterial narrowing results in clinical

symptoms. We hypothesized that iCUD have significant vascular inflammation, which is modulated by history of drug use. Therefore, using Positron Emission Tomography/Magnetic Resonance (PET/MR), we imaged the internal carotid arteries to assess atherosclerosis in 10 healthy iCUD aged 43 to 58 with cocaine lifetime use of 22.6±7.3 years and without a history of neurological or cerebrovascular disease (CVD). We compared results to findings in a sample at risk for CVD, aged 64.6±7.8. Amount of inflammation, measured with PET with 18F-fluorodeoxyglucose (18F-FDG), was calculated by the maximum arterial wall (target) to background (blood) ratio (TBR). To measure enlargement of wall area and thickness of the vessel, we used MR with 3-Dimensional dark-blood sequence. Results show that 78% of iCUD had inflamed plaque in arteries [TBRmax. (mean, SE) right (1.89, .12) left (1.7, .11); notably, TBR $\geq$ 1.6 is indicative of inflamed plaque]. Furthermore, in one sample t-tests using the comparison group's mean values, iCUD had thicker wall (mm; 1.63, .03 versus 1.27, .04, $t(8)=8.84$, $p=.00$) and larger wall area (mm2; 38.45, 1.48 versus 32.28, 1.43, $t(8)=3.34$, $p=.01$) indicating the presence of more plaque in the carotid than the much older comparison sample at risk for CVD. These PET/MR findings correlated significantly with cocaine use indices (lifetime use, craving, and addiction severity) and with nicotine and alcohol lifetime use where the more severe the drug use, the greater the carotid abnormalities ($.53 \leq r \leq .85$, $p<.01$). These preliminary results show markers of carotid disease in CVD-asymptomatic iCUD, which may exacerbate cognitive and behavioral impairments, of paramount clinical significance for combating silent disease progression.

Disclosures: **K. Bachi:** None. **V. Mani:** None. **R.Z. Goldstein:** None. **Z.A. Fayad:** None. **N. Alia-Klein:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.01/K27

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA032890

Title: Endocannabinoid system alterations in an animal model of autism spectrum disorders

Authors: ***E. S. ONAIVI**[1,1], J. ESCOSTEGUY-NETO[2], J. SANTOS-JUNIOR[2], S. SGRO[1], N. SCHANZ[1], E. DENNIS[1], L. N. PAMEN[1], C. M. LEONARD[1], K. PENKOSKI[1], M. CHUNG[1], N. TERRY[1], J. WOOD[1], S. TAMMAREDDY[1], Z. C. LIN[3], J. MORGAN[1], F. S. HALL[4], G. G. GOULD[5], B. S. BASAVARAJAPPA[6], G. R. UHL[7], S. F. ALI[8], H. ISHIGURO[9], Q.-R. LIU[1]; [1]William Paterson Univ., Wayne, NJ; [2]Federal Univ. of Sao-Paulo, Brazil, Sao-Paulo, Brazil; [3]Harvard Med. Sch., Belmont, MA; [4]Univ. of Toledo, Toledo, OH; [5]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [6]Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY; [7]New

Mexico VA Healthcare Syst., Albuquerque, NM; [8]Natl. Ctr. for Toxicology Research/FDA, Jefferson, AR; [9]Univ. of Yamanashi, Chuo-Yamanashi, Japan

Abstract: Alterations of the endocannabinoid system (ECS) are involved in the pathophysiology of neuropsychiatric disorders including autism spectrum disorders (ASDs). The interaction between genes and environmental factors including immune system dysregulation are associated with ASDs. The ECS consists of the cannabinoid receptors (CB1Rs and CB2Rs), endocannabinoids (eCBs), and the synthesizing and degradation enzymes of eCBs. ECS are involved in embryo neurodevelopment and growth and is a key regulator of the immune system via CB2Rs which are expressed on macrophages, microglial cells and neurons. We used the BTBR T+tf/J mice that have been shown to exhibit autism-like behavioral phenotypes to 1). Determine brain expression of CB2Rs throughout neurodevelopment in BTBR T+tf/J and C57BL/6J mice and also to measure the levels of eCBs, anandamide (AEA) and 2-arachidonlyl glycerol (2-AG) in frontal cortex, cerebellum and the rest of the brain by LC-MS using isotopic dilution method. 2). Evaluate the neurochemical and molecular basis of cannabinoid-induced behavioral effects and 3). Determine impact of SERT, DAT, MOR, and DAT-CI gene knock out on CBR-induced behaviors in motor function and emotionality tests. We report that CB2Rs are present and essential during neurodevelopment and its enhanced brain expression in the adult BTBR mice might be associated with the differential cannabinoid-induced behavioral effects when compared to the C57BL/6J mice. But [^{3}H] CP55,940 binding to CB1Rs did not differ between BTBR and C57BL/6J mice in the amygdala and parietal cortex. CB2R agonist, JWH133 and ACEA- CB1R agonist reduced motor activity in both BTBR and C57BL/6J mice. ACEA induced aversive behavior in both the BTBR and C57BL/6J mice while CB1R antagonist-AM251 reduced aversive behavior in both BTBR and C57Bl/6J strains. In the transgenic mice, the effect of JWH133 was genotype and gender dependent in the motor function and emotionality tests. SERT ko mice were more active in the wheel running activity (WRA) and this was enhanced by JWH133 in the male but not female SERT ko mice. Similar reductions in WRA were recorded for the male and female DAT, DAT-CI and MOR ko mice. In MOR and DAT-CI ko mice, JWH133 induced aversions but reduced aversions in SERT and DAT ko male mice in the two chamber black and white box. AEA but not 2-AG levels in the BTBR mice were reduced in the brain areas analyzed. The data indicate that dysfunction in the ECS may in part contribute to ASDs and other neuropsychiatric disorders. Further studies are required to determine the contribution of the different elements of the ECS involvement in the etiology of ASDS.

Disclosures: **E.S. Onaivi:** None. **J. Escosteguy-Neto:** None. **J. Santos-Junior:** None. **S. Sgro:** None. **N. Schanz:** None. **E. Dennis:** None. **L.N. Pamen:** None. **C.M. Leonard:** None. **K. Penkoski:** None. **M. Chung:** None. **N. Terry:** None. **J. Wood:** None. **S. Tammareddy:** None. **Z.C. Lin:** None. **J. Morgan:** None. **F.S. Hall:** None. **G.G. Gould:** None. **B.S. Basavarajappa:** None. **G.R. Uhl:** None. **S.F. Ali:** None. **H. Ishiguro:** None. **Q. Liu:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.02/K28

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant R01 DA037924

NIH/NIDA Grant R00 DA023444-03

NIMHHD 8G12 MD 007600

Title: Mechanisms and signaling downstream the cannabinoid receptor 1/beta-arrestin

Authors: *F. DELGADO-PERAZA[1], K. H. AHN[2], K. MACKIE[3], D. A. KENDALL[2], G. A. YUDOWSKI[1];
[1]Inst. of Neurobio., San Juan, PR; [2]Dept. of Pharmaceut. Sci., Univ. of Connecticut, Storrs, CT; [3]Dept. of Psychological and Brain Sci., Gill Ctr. for Biomed. Sciences, Indiana Univ., Bloomington, IN

Abstract: G protein-coupled receptors (GPCRs) are transmembrane proteins that transduce external stimulus into intracellular effector pathways. When activated by ligands, they can elicit multiple signaling cascades that are mediated by G proteins or by multifunctional scaffold proteins known as beta-arrestins. While G protein pathways are well defined, the mechanisms and pathways downstream from beta-arrestin signaling are less understood. Here, we sought to investigate the mechanisms and signaling cascades downstream from the cannabinoid 1 receptor (CB1R)/beta-arrestin. First, we tested the hypothesis that the duration of the interaction between CB1R/beta-arrestins during the endocytic process, can control beta-arrestin signaling. We characterized ligand-induced endocytosis of the CB1R in real time by total internal reflection (TIRF) microscopy at the single endocytic level in human embryonic kidney (HEK)293 cells and hippocampal neuronal cultures. Cells were transfected with the CB1R tagged with super-ecliptic phluorin (SEP). Endocytosis was initiated by bath application of different ligands and the endocytic dwell time, which is the time receptors are clustered with beta-arrestins at the endocytic pits before endocytosis, for each ligand was analyzed. We identified ligand-specific endocytic dwell times. The endogenous eicosanoid, 2-arachidonoylglycerol (2-AG), elicited prolonged dwell times (>120 seconds) and strong beta-arrestin signaling, whereas the synthetic agonist WIN 55,212-2 (WIN) elicited short ones (<120 seconds) and no beta-arrestin signaling. Furthermore, chemical inhibition of endocytosis significantly increased beta-arrestin signaling. In addition, by using antibody arrays and siRNA technology, we identified specific signaling pathways controlled by beta-arrestins. Our results indentify the signaling network downstream from CB1R/beta-arrestins and propose a molecular mechanism controlling this type of signaling. Furthermore, we propose modulation of receptor trafficking as a novel approach to control beta-arrestin mediated signaling.

Disclosures: F. Delgado-Peraza: None. **K.H. Ahn:** None. **K. Mackie:** None. **D.A. Kendall:** None. **G.A. Yudowski:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.03/K29

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA032194

Mayo Foundation for Medical Education and Research

Mayo Graduate School

Title: Elucidating cannabinoid biology in zebrafish

Authors: *R. G. KRUG, M. O. PETERSEN, K. J. CLARK;
Mayo Clin., Rochester, MN

Abstract: Although the number of annual cannabis users exceeds 100,000,000 globally and an estimated 9% of these individuals will suffer from dependency, a dearth of knowledge exists about the potential consequences on public health. However, the psychoactive constituents of cannabis are known to signal through the endocannabinoid (eCB) system, and to disrupt features of vertebrate physiology and behavior. While studies have revealed that the eCB anandamide (AEA) regulates stress response system (SRS) activity, little is known about the pathological consequences of disrupted AEA signaling. Our central hypothesis is that disruptions in the AEA signaling system have pathological consequences on vertebrate behavior and physiology, including dysregulation of the SRS. Herein, we use a preclinical zebrafish model to clarify the ramifications of disturbances in the AEA signaling system. Using qRT-PCR and *in situ* hybridization we show that the genes encoding enzymes that synthesize (*abhd4*, *gde1*, *napepld)*, enzymes that degrade (*faah*, *faah2a*, *faah2b*), and receptors that bind (*cnr1*, *cnr2*, *gpr55-like*) AEA are expressed throughout development. We show that disruptions of this system via exogenous cannabinoid administration results in altered behavior and physiology, including increased secretion of glucocorticoids in our stress response reporter line. We are developing a zebrafish AEA signaling mutant library using transcription activator-like effector nucleases (TALENs). Currently, we are identifying our first mutant lines and will share the preliminary results of behavioral assays using our first mutants. Collectively, these results establish zebrafish as a viable model for studying AEA signaling, and lay a foundation for informing a better understanding of the toxicological and therapeutic potential of the eCB system.

Disclosures: R.G. Krug: None. **M.O. Petersen:** None. **K.J. Clark:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.04/K30

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH - Government employee duty

Title: Cannabinoid cb1 and cb2 receptors mediate the classical tetrad effects of delta9-tetrahydrocannabinol in mice

Authors: *Z. XI[1], X.-F. WANG[2], Y. HE[2], G. BI[2], E. GARDNER[2];
[1]NIDA, IRP, Baltimore, MD; [2]NIDA, Baltimore, MD

Abstract: In animals, cannabinoid agonists such as delta9-THC produce a characteristic combination of tetrad symptoms - hypothermia, analgesia, hypoactivity, and catalepsy. However, the receptor mechanisms underlying these actions are incompletely understood. When cannabinoid CB1 and CB2 receptors were first cloned in 1990s, CB1 receptor was found in the brain and periphery, while CB2 receptor was found only in periphery. Therefore, it is believed that brain CB1, not CB2, receptor mediates the action produced by cannabis. This view has been challenged by recent finding that functional CB2 receptor is expressed in the brain and in midbrain dopamine neurons. This new finding inspired us to re-examine whether brain CB2 receptor is also involved in the action produced by cannabis such as delta9-THC. To address this issue, we first compared the behavioral response to delta9-THC under the same experimental conditions between wild-type (WT) and CB1 receptor-knockout (CB1-KO) or CB2 receptor-knockout mice (CB2-KO). We found that delta9-THC, at 10 or 30 mg/kg (i.p.), produced dose-dependent analgesia (as assessed by hot-plate test), hypothermia, catalepsy and rotarod performance impairment in WT mice, but not in CB1-KO mice. Surprisingly, deletion of CB2 receptor in CB2-KO mice also blunted delta9-THC-induced analgesia and catalepsy. We then observed the effects of the selective CB1 receptor agonist (ACEA) or CB2 receptor agonist (JWH133) in WT mice. We found that, systemic administration of ACEA (1-10 mg/kg, i.p.) or JWH133 (1-10 mg/kg, i.p.) alone failed to produce significant effects in the above measurements, while co-administration of ACEA and JWH133 produced significant analgesia, hypoactivity and catalepsy. These findings suggest that 1) brain CB1 receptor plays a predominant role in mediating delta9-THC-induced tetrad effects; 2) brain CB2 receptor also play an important role in mediating delta9-THC-induced analgesia and catalepsy; and 3) co-activation of brain CB1 and CB2 receptors are required in mediating the behavioral effects produced by cannabinoid ligands. (Supported by NIDA IRP)

Disclosures: **Z. Xi:** None. **X. Wang:** None. **Y. He:** None. **G. Bi:** None. **E. Gardner:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.05/K31

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant 25267

NIDA Grant 19222

Title: Differential tolerance produced by daily administration of Δ^9-THC and JWH-018 in rhesus monkeys

Authors: *M. L. COCKE, L. R. MCMAHON;
Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San A, San Antonio, TX

Abstract: The *Cannabis* derivative Δ^9-tetrahydrocannabinol (Δ^9-THC) and the synthetic cannabinoid naphthalen-1-yl-(1-pentylindol-3-yl) methanone (JWH-018) are both CB_1 receptor agonists; however, unlike Δ^9-THC, JWH-018 has been linked to adverse effects such as seizures and hypertension. The disparity between adverse effects could be due to JWH-018 having higher CB_1 receptor efficacy than Δ^9-THC, evidenced most commonly by greater maximum stimulation of inhibitory G proteins. In a previous study, daily Δ^9-THC treatment produced tolerance and cross-tolerance to JWH-018 in a non-human primate model of subjective effects; however, tolerance to Δ^9-THC was greater than cross-tolerance to JWH-018, consistent with a difference in CB_1 receptor efficacy. What remains unclear is the extent to which daily JWH-018 administration alters sensitivity to JWH-018. This study tested the hypothesis that tolerance to Δ^9-THC is greater than tolerance to an equally effective dose of JWH-018, as would be predicted for chronic treatment with a low versus a high efficacy agonist. Rhesus monkeys (*Macaca mulatta*) discriminated Δ^9-THC (0.1 mg/kg i.v.) from vehicle under an FR5 schedule of lever pressing to avoid a noxious stimulus. Both Δ^9-THC and JWH-018 produced dose-dependent increases in Δ^9-THC lever responding to 100%; the respective ED_{50} values were 0.026 and 0.0084 mg/kg, a difference of 3-fold. The time courses of discriminative stimulus effects following subcutaneous administration were compared: Δ^9-THC (1 mg/kg) produced 80% Δ^9-THC lever responding for 8-12 h, whereas the duration of action of JWH-018 (0.32 mg/kg) was 4-8 h. According to these time courses, Δ^9-THC (1 mg/kg s.c.) was administered once daily and JWH-018 (0.32 mg/kg s.c.) was administered twice-daily 6 h apart. Δ^9-THC treatment produced a 3.8-fold loss of sensitivity after three days and a 16-fold loss of sensitivity after 7 days. JWH-018 treatment produced a 2.1-fold loss of sensitivity after three days and a 4.7-fold loss of sensitivity after 7 days. Consistent with the hypothesis, tolerance to the low efficacy CB_1 receptor agonist Δ^9-THC was greater than tolerance to the high efficacy CB_1 receptor agonist JWH-018. The differential development of tolerance among CB_1 receptor agonists as a function of efficacy might underlie the greater incidence of adverse effects following use of synthetic cannabinoids as compared with *Cannabis*.

Disclosures: **M.L. Cocke:** None. **L.R. McMahon:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.06/K32

Topic: B.07. Synaptic Transmission

Support: NIH/NIMH R01 MH081164-01A2

Title: Increased cortical inhibition in autism-linked neuroligin 3 r451c mice is due to loss of tonic endocannabinoid signaling

Authors: ***H. E. SPEED**[1], I. MASIULIS[2], J. GIBSON[3], C. POWELL[4];
[1]Neurol., UT Southwestern Med. Ctr., Dallas, TX; [2]Quantitative Morphology Core, [3]Neurosci., [4]Neurol., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: A single, maternally inherited, X-linked point mutation leading to an arginine to cysteine substitution at amino acid 451 (R451C) of Neuroligin 3 is a likely cause of autism in two brothers. Knockin mice expressing the $NL3^{R451C}$ mutation in place of wild-type NL3 demonstrate increased inhibitory synaptic strength in somatosensory cortex, resulting in an excitatory/inhibitory (E/I) imbalance that is potentially relevant for autism-associated behavioral deficits characteristic of these mice. We have replicated the increase in evoked inhibitory postsynaptic currents (eIPSCs) onto layer II/III cortical pyramidal neurons. We also find that increased frequency of spontaneous IPSCs in $NL3^{R451C}$ mice occurs in the absence of action potential-driven transmission. This suggests the E/I imbalance is due to changes at the synapse level, as opposed to the network level. Next, we use paired whole-cell recordings in an attempt to identify specific interneuron subtypes affected by the $NL3^{R451C}$ mutation. Curiously, we observe no change in the amplitude of cell-to-cell, unitary IPSCs from parvalbumin-positive (PV) or somatostatin-positive (SOM) interneurons onto pyramidal neurons. We also observe no change in the number or density of PV and SOM interneurons in LII/III of somatosensory cortex. This effectively rules out a role for these particular interneurons in the increased inhibitory synaptic transmission, pointing to perhaps alternative interneuron subtypes. Lastly, impaired endocannabinoid signaling has been implicated in hippocampal synaptic dysfunction in $NL3^{R451C}$ mice, but has not been investigated at cortical synapses. We find that bath application of the CB1 antagonist, AM 251 in WT mice eliminates the $NL3^{R451C}$ increase in evoked IPSC amplitude and mIPSC frequency, indicating that increased inhibitory transmission in mutant mice is due, at least in part, to a loss of tonic endocannabinoid signaling through CB1 receptors likely acting at interneurons other than PV or SOM.

Disclosures: **H.E. Speed:** None. **I. Masiulis:** None. **J. Gibson:** None. **C. Powell:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.07/K33

Topic: C.17. Drugs of Abuse and Addiction

Support: DA036385

Pennsylvania Department of Health using Tobacco CURE Funds

Title: Morphine and ethanol reward, tolerance, and dependence in mice expressing a desensitization-resistant form of the cannabinoid receptor 1 (CB1)

Authors: M. L. ZEE[1], D. J. MARCUS[2], M. GONEK[3], A. H. REDMOND[1], *D. J. MORGAN[1]; [1]Dept. of Anesthesiol., Penn State Col. of Med., Hershey, PA; [2]Vanderbilt Univ., Nashville, TN; [3]Virginia Commonwealth Univ., Richmond, VA

Abstract: The focus of this study was to investigate whether enhanced endocannabinoid system (ECS) signaling modulates reward, tolerance, and dependence for morphine and ethanol. Preference and consumption of ethanol is attenuated in CB1 knockout mice using a two-bottle choice voluntary drinking paradigm. Studies also demonstrate involvement of the CB1 receptor in the modulation of reward, dependence and tolerance to morphine. We produced S426A/S430A mutant mice expressing a desensitization-resistant form of CB1 to study the importance of desensitization for tolerance to cannabinoid agonists. During the characterization of these novel mutant mice, we found that they also display an exaggerated and prolonged acute response to Δ9-THC and endogenous endocannabinoids. Given the important role of CB1 in modulating the effects of opioid and ethanol reward and dependence, we decided to use the S426A/S430A mutant mice as a novel model to examine the effect of enhanced ECS signaling on these processes. Ethanol intake was measured using a 24-hour, continuous-access voluntary drinking paradigm. We found that S426A/S430A mice consumed significantly more 6 and 9% ethanol. Tolerance to ethanol-induced ataxia was examined using the rotarod, while tolerance to morphine-induced antinociception was assessed using the hotplate and tail-flick tests. Despite differences in ethanol intake, S426A/S430A and wild-type mice exhibit equivalent ethanol and morphine tolerance. Likewise, S426A/S430A also develop normal and robust CPP for morphine and cocaine. Morphine dependence was determined by counting naloxone-precipitated withdrawal symptoms in mice implanted with 75mg morphine pellets. Both genotypes displayed similar severity of morphine dependence; however, we found that S426A/S430A mutants recovered from precipitated withdrawal more quickly than wild-type littermates. These findings suggest that CB1 modulation of reward for, tolerance to, and dependence on ethanol and morphine is not heavily impacted by elevated ECS sensitivity due to disruption of CB1 desensitization. Although the S426A/S430A mice may indeed possess enhanced sensitivity to

exogenously administered cannabinoids, our data suggests that they have limited utility for drug addiction research.

Disclosures: **M.L. Zee:** None. **D.J. Marcus:** None. **M. Gonek:** None. **A.H. Redmond:** None. **D.J. Morgan:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.08/K34

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Grant 021142 (SEB)

TTUHSC Grant 121035 (JG)

Title: Antinociceptive effects of synthetic tetracycline compound and influence of Cx3cr1 in inflammatory pain

Authors: *J. GUINDON, B. SEEGMILLER, C. BEZBORUAH, P. C. MARQUADT, J. M. MARTINEZ, S. E. BERGESON;
Dept. of Pharmacol. and Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Tetracycline compounds are broad-spectrum antibiotics used against different bacterial infectious diseases. Synthetic tetracycline compounds have been developed and are able to reduce infection as well as inflammation. However, the effect on alleviation of pain, and more specifically reduction of inflammatory pain, has not been investigated. The goal of the study is to evaluate the effect of synthetic tetracycline compounds using an inflammatory pain model and mechanical and cold allodynia responses. The formalin test (10 µl at 2.5 % intraplantar) was use to evaluate pain threshold differences between male wild-type C57BL/6 mice and male GFP Cx3cr1 +/- mice. First, our preliminary data demonstrate that male GFP Cx3cr1 +/- mice show lower pain threshold in both (acute and inflammatory) phases of the formalin test. Second, we evaluated the effect of a synthetic tetracycline compound on inflammatory pain and on mechanical and cold allodynia. Our data indicate that mechanical and cold allodynia remains unchanged after administration of a synthetic tetracycline compound meaning values are similar to baseline levels. However, synthetic tetracycline compound lower pain threshold in both phases of the formalin test from 4 hours up to 20 hours after its administration and showed antinociceptive effects to mechanical and cold allodynia following the formalin test (60 minutes after injection of formalin). Moreover, mechanical and cold allodynia responses return to baseline levels 2 hours after the formalin test. These results demonstrate potential antinociceptive properties of synthetic tetracycline compound with long lasting effect up to 20 hours after its administration. Further studies are needed to investigate the mechanism underlying this

antinociceptive effect of synthetic tetracycline compounds. We will also evaluate potential stronger antinociceptive effect of the synthetic tetracycline compound in Cx3cr1 +/- male mice and evaluate if there is any gender specific differences. Antinociceptive properties of synthetic tetracycline compound could be a great alternative avenue to relieve inflammatory pain and improve alleviation of pain in patients.

Disclosures: J. Guindon: None. **B. Seegmiller:** None. **C. Bezboruah:** None. **P.C. Marquadt:** None. **J.M. Martinez:** None. **S.E. Bergeson:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.09/K35

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA036385

NIH Grant DA037355

TTUHSC Grant 121035

Title: Tolerance to the antinociceptive effects of Δ9-THC in the formalin model of inflammatory pain

Authors: *M. B. YUILL[1], D. J. MORGAN[2,3], J. GUINDON[4];
[2]Dept. of Anesthesiol., [1]Penn State Hershey Col. of Med., Hershey, PA; [4]Dept. of Pharmacol. and Neurosci., [3]Texas Tech. Univ. Hlth. Sci. Ctr. Sch. of Med., Lubbock, TX

Abstract: The use of cannabinoids to manage pain is of interest given the rise of opiate abuse among the general population. Δ^9-THC produces potent antinociceptive effects but, like opiates, is subject to tolerance. The objective of this study was to examine the complete time-course of Δ^9-THC tolerance in a model of inflammatory pain. The effect of Δ^9-THC was assessed in male wild-type C57BL/6 mice subjected to inflammatory pain using formalin (10 µl at 2.5 % intraplantar). Experimental groups were subjected to the formalin test after receiving chronic daily injections of Δ^9-THC (6 mg/kg), or vehicle, for periods of time starting from zero consecutively up to eight days. We also examined the effect of JNK inhibitor SP600125 on Δ^9-THC. Control groups injected with saline for 1, 4, and 8 days were examined. Mice tested after one day of exposure showed the greatest level of antinociception while mice treated for eight days showed almost no antinociception. Preliminary data suggest that co-administration of SP6 (3 mg/kg) with Δ^9-THC prolongs the onset of full tolerance from 8 to 12 days. In phase I (acute pain), tolerance develops gradually across eight days, with the largest jump between two and three days of chronic administration. While also gradual across eight days, in phase II of the

formalin test, the largest increase seems to occur between three and four days of chronic administration. These results are consistent with the findings in other models such as acute pain showing onset of tolerance by three days of daily administration. Further studies are needed to investigate the longer retained efficacy of Δ^9-THC appearing in inflammatory pain relative to acute pain in the tail-flick test. The findings of this study reinforce the validity of the formalin model as a pathologically relevant tool to assess cannabinoid tolerance in mice models. Also, it provides a detailed day-by-day map of the progression of Δ^9-THC tolerance. Preliminary results also suggest that JNK signaling is involved in this observed tolerance. Acknowledgements: Funded by NIH grants DA036385 (DJM), DA037355 (DJM), and funded by Texas Tech University Health Sciences Center School of Medicine grant 121035(JG).

Disclosures: **M.B. Yuill:** None. **D.J. Morgan:** None. **J. Guindon:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.10/K36

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/ORWH 5P50 DA016511-12

Burroughs Wellcome Fund Postdoctoral Enrichment Fellowship

DA015369

DA003906

DA037722

Title: Development of a novel rodent model of THC self-administration

Authors: *S. M. SPENCER[1], C. GARCIA-KELLER[2], M. D. SCOFIELD[2], N. ALLEN[2], D. SCHWARTZ[2], P. W. KALIVAS[2];
[1]Neurosci., [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: Cannabis is the most frequently used illicit drug worldwide, but preclinical research on its effects has been hampered by lack of an animal model since rats will not maintain self-administration of isolated Δ^9-tetrahydrocannabinol (THC), the drug's main psychoactive component. THC on its own may produce unpleasant side effects including increased anxiety, but cannabis contains over 60 cannabinoids and more than 400 additional chemicals. Cannabidiol (CBD), a non-psychoactive cannabinoid, may counter some of the aversive properties of THC by producing anxiolytic and anti-psychotic effects. Thus in the present investigation, one strategy that was employed to promote drug taking in male Sprague-Dawley

rats was by combining CBD with THC in a ratio of 10:1, a proportion previously demonstrated to neutralize a THC conditioned place aversion. Moreover, we used a Volcano vaporizer to pre-expose rats to THC:CBD vapor (10 mg THC vapor per pad) for 5 days prior to initiating intravenous THC:CBD self-administration (4 g/kg/0.05 ml infusion) as it has previously been demonstrated that THC pre-exposure facilitates formation of a THC conditioned place preference rather than aversion. We determined that our THC vapor pre-exposure provided a physiologically relevant dose of THC as we were able to measure a decrease in core body temperature after exposure. During self-administration, clear lever discrimination was observed with greater than 2-fold preference for the drug-associated lever. The rats sustained low levels of responding with an average of 6 infusions per 2-hr session although there was high inter-individual variability. We generated a dose response curve in (1.27, 4, and 12.64 g/kg) and found that highest responding was observed at 4 g/kg/infusion. Importantly, we also demonstrated both cue-induced and THC-primed (1 mg/kg, ip) reinstatement in animals extinguished from THC:CBD. This affords the opportunity to initiate a reverse-translational investigation of n-acetylcysteine (NAC) to test its effects on reinstatement as this drug has recently demonstrated efficacy in clinical studies of marijuana dependence. Additional studies will examine biomarkers of altered glutamatergic synaptic plasticity in the nucleus accumbens after THC self-administration. In summary, we have established a rodent model of THC self-administration allowing us to evaluate THC-dependent brain changes relevant to addiction and relapse.

Disclosures: S.M. Spencer: None. **C. Garcia-Keller:** None. **M.D. Scofield:** None. **N. Allen:** None. **D. Schwartz:** None. **P.W. Kalivas:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.11/K37

Topic: C.17. Drugs of Abuse and Addiction

Title: Cannabinoid withdrawal-induced adaptations in the habenulomesencephalic pathway

Authors: *A. MUNTONI[1], S. ARONI[2], C. SAGHEDDU[2], M. PISTIS[2,1];
[1]CNR Neurosci. Institute-Cagliari, Monserrato, Italy; [2]Dept. of Biomed. Sciences, Div. of Neurosci. and Clin. Pharmacol., Univ. of Cagliari, Cagliari, Italy

Abstract: The mesolimbic reward system arising from dopamine (DA) neurons of the midbrain ventral tegmental area (VTA) shows a profound reduction in its function during cannabinoid withdrawal. This hypoactivity, a common feature of several abused drugs, is thought to underlay the withdrawal-induced aversive affective states eventually leading to compulsive drug seeking and relapse. The lateral habenula (LHb) exerts a negative control over the VTA via the GABA rostromedial tegmental nucleus (RMTg), encoding aversion-related stimuli. In fact, both RMTg

and LHb cells are activated by negative/unpleasant events, and inhibited by rewarding/positive stimuli. Therefore, these nuclei represent a potential convergence point for drug-evoked reward and aversive opponent processes. On these bases, we hypothesized that the LHb-RMTg pathway might be causally involved in the hypodopaminergic state which occurs during cannabinoid withdrawal. To address this question, we performed single unit extracellular recordings from either VTA, RMTg and LHb neurons in anesthetized male Sprague-Dawley rats. To induce Δ^9-tetrahydrocannabinol (Δ^9-THC) dependence, rats were chronically treated (15 mg/kg, i.p.) twice daily for 6.5 days. Administration of the cannabinoid antagonist rimonabant (5 mg/kg, i.p.) precipitated a robust behavioural withdrawal syndrome, while abrupt Δ^9-THC suspension caused milder signs of abstinence. Electrophysiological experiments confirmed that Δ^9-THC withdrawal is accompanied by a marked decrease in the discharge frequency and burst firing of VTA DA neurons. Remarkably, in Δ^9-THC withdrawn rats the duration of RMTg-evoked inhibition lasted longer than controls. In contrast, the spontaneous activity of RMTg GABA neurons was reduced in cannabinoid-withdrawn rats. Consistent with results, we also found that the firing rate of RMTg-projecting LHb neurons was strongly depressed during cannabinoid withdrawal. These data support the hypothesis that enhanced GABA inputs from the RMTg might contribute to the hypodopaminergic state induced by cannabinoid withdrawal. They also confirm that functional changes in the habenulomesencephalic circuit are implicated in the mechanisms underlying drug dependence and addiction.

Disclosures: **A. Muntoni:** None. **S. Aroni:** None. **C. Sagheddu:** None. **M. Pistis:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.12/K38

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS070715

NIH Grant DA030404

Title: Modulation of CB1 cannabinoid receptor signaling and adaptation by D2 dopamine receptors

Authors: D. E. SELLEY[1], L. S. MIDDLETON[1], J. J. BURSTON[2], D. K. GRANDY[3], *L. J. SIM-SELLEY[1];
[1]Virginia Commonweatlh Univ., Richmond, VA; [2]The Univ. of Nottingham, Nottingham, United Kingdom; [3]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Gi/o-coupled CB1 receptors (CB1R) in striatum produce motor suppression and catalepsy and regulate motivation. Repeated CB1 agonist administration produces tolerance that

is associated with CB1R desensitization and downregulation in the CNS, however CB1Rs in striatal circuits are somewhat resistant to these adaptations. CB1Rs co-localize with D2 receptors (D2R) on striatopallidal medium spiny neurons, and interaction between these receptors affects their activity and expression. CB1R and D2R can heteromerize, which switches their G-protein coupling from Gi/o to Gs. We examined D2R-CB1R interactions in G-protein activation using [35S]GTPγS binding in wild-type (WT) or D2R knockout (KO) mice and CHO cells stably expressing CB1R (CB1-CHO) or CB1 and D2R (CB1/D2-CHO). Maximal CB1R mediated G-protein activation was reduced in both dorsal and ventral striatum of D2R KO compared to WT mice. In dorsal striatum of WT but not D2R KO mice, incubation with both the CB1 agonist WIN55,212-2 (WIN) and D2 agonist quinelorane (Quin) produced less G-protein activation than WIN alone. Despite reduced CB1R mediated G-protein activation in D2R KO mice, adenylyl cyclase (AC) inhibition by WIN was enhanced in dorsal and unchanged in ventral striatum of D2R KO mice, suggesting that D2R might switch a subset of CB1Rs from Gi to Gs protein activation. Interestingly, maximal G-protein activation by D2R did not differ between CB1R KO and WT mice in dorsal or ventral striatum, but the Quin EC50 value in dorsal striatum was greater in CB1R KO mice. Because D2R alters CB1R signaling, co-expression with D2R might contribute to resistance of striatal CB1R to agonist-induced adaptation. This was tested in a model cell system. CB1-CHO and CB1/D2-CHO cells were treated for 24 hr with WIN or Quin alone or in combination. WIN treatment desensitized CB1R mediated G-protein activation in CB1-CHO cells without downregulating CB1Rs. Importantly, CB1R desensitization by WIN was reduced in CB1/D2-CHO cells, but co-treatment with WIN + Quin did not further reduce CB1R desensitization. Treatment of CB1-CHO cells with WIN + the AC activator forskolin attenuated CB1R desensitization compared to WIN alone, suggesting that heteromer coupling to Gs could play a role in D2R mediated attenuation of CB1R desensitization. Quin treatment of CB1/D2-CHO cells desensitized D2R, but enhanced CB1R mediated G-protein activation, whereas WIN treatment did not affect G-protein activation by D2R. These results demonstrate asymmetric interactions between CB1R and D2R in G-protein signaling and receptor adaptation.

Disclosures: **D.E. Selley:** None. **L.S. Middleton:** None. **J.J. Burston:** None. **D.K. Grandy:** None. **L.J. Sim-Selley:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.13/K39

Topic: C.17. Drugs of Abuse and Addiction

Title: Involvement of endocannabinoid system on cognitive dysfunction during withdrawal of repeated methamphetamine administration in mice

Authors: R. FUKUMORI, S. YAMADA, *T. YAMAGUCHI, T. YAMAMOTO;
Nagasaki Intl. Univ., Sasebo, Nagasaki, Japan

Abstract: Repeated administration of methamphetamine causes reverse tolerance and/or behavioral sensitization in mice. However, the cognitive deficits induced by withdrawal after repeated methamphetamine administration have not been sufficiently studied until now. On the other hand, endocannabinoid systems play important roles in physiological functions in the central nervous system, such as pain perception, appetite, psychomotor behavior, emotion, reward system and cognitive function. Furthermore, we previously reported that the involvement of cannabinoid CB1 receptors in the reinstatement of methamphetamine-seeking behaviors in rats. In this study, we investigated relationship between cognitive deficits and development of behavioral sensitization by using the cannabinoid CB1 receptor knockout mice. Mice were subcutaneously administered methamphetamine at dose of 0.1-1.8 mg/kg or saline, every other day for 30 days (15 injections). In wildtype mice, locomotor activity was enhanced by the repeated administration of methamphetamine at dose of 1.0 and 1.8 mg/kg. 10 or 30 days after withdrawal, the mice were tested cognitive functions. In novel object recognition test, approach time to novel object has decreased during withdrawal of repeated 1.8 mg/kg methamphetamine administration. In addition, prepulse inhibition had been suppressed during withdrawal of repeated methamphetamine administration at dose of 1.0 and 1.8 mg/kg. On the other hand, in cannabinoid CB1 receptor knockout mice, the locomotor activity was not enhanced by repeated administration of methamphetamine. Furthermore, cannabinoid CB1 receptor knockout mice were not shown dysfunctions of cognition and prepulsee inhibition by repeated administration of methamphetamine. Our data suggest that the cannabinoid CB1 receptor is involved in the development of behavioral sensitization and cognitive/sensorimotor gating deficits during withdrawal of repeated methamphetamine administration.

Disclosures: R. Fukumori: None. **S. Yamada:** None. **T. Yamaguchi:** None. **T. Yamamoto:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.14/K40

Topic: C.17. Drugs of Abuse and Addiction

Support: NDA/IRP

Title: Cocaine self-administration up-regulates cannabinoid CB_2 gene expression in mouse brain

Authors: *H. ZHANG[1], Q.-R. LIU[1], G.-H. BI[1], R. CHANDRA[2], M. LOBO[2], E. GARDNER[1], Z.-X. XI[1];

[1]NIDA/IRP, Baltimore, MD; [2]Dept. of Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: We have recently reported that brain cannabinoid CB_2 receptors (CB_2Rs) regulate midbrain dopamine (DA) neuronal activity, nucleus accumbens DA release and intravenous cocaine self-administration in mice and rats (Xi et al., Nature Neuroscience, 2011; Zhang et al., PNAS, 2014). These findings appear to conflict with anatomic evidence that brain CB2 gene level is very low (~50-fold) compared to that in the periphery - e.g., spleen. We hypothesized that acute or chronic use of drugs of abuse may up-regulate brain CB2 receptor expression, and produce significant effects on brain function. To test this hypothesis, we first treated animals with a single injection or repeated injections of cocaine, or chronic cocaine self-administration, respectively, and then measured brain CB2 mRNA levels using quantitative real-time PCR (qRT-PCR) and *in situ* hybridization (ISH) assays. We found that: 1) chronic intravenous cocaine self-administration (1 mg/kg/infusion × 50 infusions/day × 4 weeks) significantly up-regulated (4-5 fold) CB_2 mRNA expression in the prefrontal cortex (PFC) and striatum of mice compared to that observed in oral sucrose self-administration (control) mice or drug naïve mice. In contrast, a single injection (10, 20, 30 mg/kg, i.p.) or repeated injections of cocaine (10 mg/kg, i.p. per day, for 7 days) (i.e. locomotor sensitization dose regimen) failed to alter brain CB_2 mRNA expression as assessed by qRT-PCR; 2) ISH assays show similar findings - CB_2 mRNA is significantly up-regulated in cortical and striatal neurons as well as VTA DA neurons. To determine the cell types of striatal GABAergic medium-spiny neurons (MSNs) expressing CB2Rs, we used D1- versus D2-eGFP transgenic mice and fluorescence activated cell sorting (FACS) techniques, and found that CB2 mRNA is mainly expressed in D2-MSNs (3~4-fold higher in D2-MSNs than in D1-MSNs) in normal subjects. Repeated cocaine administration (20 mg/kg, i.p. per day for 7 days) significantly up-regulated CB2 mRNA expression in D1-MSNs, but not in D2-MSNs. Taken together, these findings suggest that brain CB_2 receptors are inducible and responsive to chronic cocaine abuse or repeated large doses of cocaine. Thus, these findings not only well explain the anti-addictive effects of CB2R agonists observed in cocaine self-administration mice, but also suggest that brain CB_2Rs may constitute a new target in medication development for the treatment of drug addiction and possible other CNS disorders.

Disclosures: H. Zhang: None. **Q. Liu:** None. **G. Bi:** None. **R. Chandra:** None. **M. Lobo:** None. **E. Gardner:** None. **Z. Xi:** None.

Poster

318. Cannabinoids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 318.15/K41

Topic: C.17. Drugs of Abuse and Addiction

Support: Bank of Sardinia Foundation, Grant 2014

Title: Elevation of kynurenic acid levels suppresses Δ9-tetrahydrocannabinol-induced excitation of mesolimbic dopamine and prefrontal cortex pyramidal neurons

Authors: *M. PISTIS[1,2], M. MELIS[1], A. MUNTONI[2], C. SAGHEDDU[1];
[1]Univ. of Cagliari, Monserrato, Italy; [2]C.N.R. Neurosci. Inst., Cagliari, Italy

Abstract: Δ9-tetrahydrocannabinol (THC), the major psychoactive component of Cannabis extracts, like most drugs of abuse, enhances dopaminergic (DA) transmission by increasing both DA neuron firing rate and DA release in the nucleus accumbens shell (shNAc), an effect that underlies the rewarding and dependence-inducing effects of marijuana. We have recently demonstrated that elevations of brain levels of kynurenic acid (KYNA), an endogenous product of the normal metabolism of amino acid L-tryptophan, suppresses THC-induced behavioral and neurochemical effects, in rats and monkeys [1]. On these bases, we carried out *in vivo* electrophysiological single cell recordings in anesthetized rats to investigate how KYNA modulates THC-induced electrophysiological actions on DA neurons in the ventral tegmental area (VTA) and pyramidal neurons in the medial prefrontal cortex (mPFC). Neurons were selected as projecting to the shNAc by antidromic stimulation. According with previous studies, THC (0.3-2.4 mg/kg), increased firing activity of DA (137.1 ± 4.1 %) and mPFC (306.2 ± 75.6 %) cells projecting to the shNAc. To enhance brain levels of KYNA, the kynurenine-3-monooxygenase inhibitor, Ro 61-8048 (Ro, 30 mg/kg, i.p.) was administered 40 minutes before recordings. Consistent with microdialysis and behavioral studies, THC-induced increase in firing activity was completely abolished in DA (103.6 ± 3.3 %) as well in mPFC (119.4 ± 28.1) cells recorded from rats pretreated with Ro. *Ex vivo* patch clamp experiments confirmed that KYNA prevents THC-induced depression of excitatory post-synaptic potentials in DA neurons. KYNA was suggested to act as a negative allosteric modulator of α7 nicotinic acetylcholine receptors (α7-nAChRs), therefore, to prevent Ro effects we administered a positive allosteric modulators of α7-nAChRs, PNU120596. PNU120596 partially prevented the effects of Ro on mPFC pyramidal neurons, suggesting that the electrophysiological effects of KYNA might be dependent on α7-nAChR. The involvement of α7-nAChR was confirmed also with patch clamp experiments. Patients seeking help for Cannabis dependence are increasing worldwide but specific pharmacological treatments are lacking. Together with recent neurochemical and behavioral studies, our results support the hypothesis that specific modulation of KYNA levels might represent an innovative therapeutic approach to treat Cannabis dependence. Reference Justinova Z, Mascia P, Wu HQ, Secci ME, Redhi GH, et al. (2013) Reducing cannabinoid abuse and preventing relapse by enhancing endogenous brain levels of kynurenic acid. Nature Neuroscience 16: 1652-1661.

Disclosures: **M. Pistis:** None. **M. Melis:** None. **A. Muntoni:** None. **C. Sagheddu:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.01/K42

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA-IRP

Title: The novel dopamine D3 receptor antagonists CAB02-015 and BAK4-54 inhibit oxycodone self-administration and reinstatement of drug-seeking behavior in rats

Authors: *Z.-B. YOU, G.-H. BI, C. BOATENG, A. BANALA, E. E. GARDNER, Z.-X. XI, A. H. NEWMAN;
Mol. Targets and Medications Discovery Br., NIDA-IRP/NIH/DHHS, Baltimore, MD

Abstract: The use of prescribed opioid analgesics such as oxycodone has been increased dramatically in recent years, which parallels the increases in prescription opioid abuse and drug-related deaths worldwide. Thus, understanding the rewarding properties of prescription opioids, and accordingly, developing effective pharmacotherapies for treatment of prescription opioid abuse has become a critical matter of public health. In the present study, we studied the rewarding and reinforcing properties of oxycodone in animal models of drug addiction, and the potential roles of the novel dopamine D3 receptor antagonists CAB02-015 or BAK4-54 in mediation of these effects in rats. We have found that animals rapidly acquired oxycodone self-administration in a dose range of 0.03 -0.24mg/kg/infusion, with a pattern that is similar to those of heroin or cocaine. Pretreatments in drug trained rats with either CAB02-015 or BAK4-54 (0.4~10mg/kg, i.p.) inhibited oxycodone self-administration dose-dependently. In addition, repeated treatments with CAB02-015 (0.4, 4 mg/kg, i.p. for 7 days) during the extinction sessions (in which oxycodone was replaced by saline) dose-dependently accelerated the extinction of drug-seeking behavior (as indicated by the accelerated decline in responding on the lever previously associated with oxycodone in CAB02-015 versus vehicle treated groups) and inhibited reinstatement of oxycodone-seeking induced by oxycodone priming injection (1 mg/kg, i.p.). Taken together, these findings indicate that 1) oxycodone possesses similar addictive properties as heroin and cocaine; and 2) the novel D3 receptor antagonists CAB02-015 and BAK4-54 may have therapeutic potential for addiction treatment associated with prescription opioid use.

Disclosures: Z. You: None. **G. Bi:** None. **C. Boateng:** None. **A. Banala:** None. **E.E. Gardner:** None. **Z. Xi:** None. **A.H. Newman:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.02/L1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA IRP

DoD Contract PR110146

Title: Effects of the non-opioid (+)-naltrexone and the peripherally active (+)-N-methylnaltrexone in rats self-administering the mu agonist remifentanil

Authors: C. ZANETTINI[1], T. HIRANITA[2], *L. R. WATKINS[1], B. R. SELFRIDGE[3], K. C. RICE[3], J. L. KATZ[2];
[1]Dept Psychology & Neurosci., Univ. Colorado At Boulder, Boulder, CO; [2]Psychobiology, NIDA Intramural Res. Program, Baltimore, MD; [3]Drug Design and Synthesis Section, NIDA Intramural Res. Program, Rockville, MD

Abstract: Toll-like receptor 4 (TLR4) is a protein that detects lipopolysaccharide from gram-negative bacteria and is involved in activation of innate immune responses to foreign substances. TLR4 is expressed centrally and peripherally. It was recently reported that the non-opioid TLR4 antagonist, (+)-naloxone, decreases self-administration of remifentanil, a short acting mu-opioid agonist, in rats, suggesting an involvement of TLR4 in the reinforcing effects of mu-opioids (J. Neurosci. 32: 11187). Using the CNS-active and peripherally acting TLR4 antagonists (+)-naltrexone and (+)-N-methylnaltrexone, respectively, the present study compared i) central vs peripheral components of this effect, and ii) the specificity of the effect by comparing effects on responding maintained under comparable schedules of remifentanil or cocaine injection, and food presentation. One group of rats was trained to self-administer remifentanil (0, 0.1-3.2 µg/kg/inj, IV) under a fixed ratio 5-response schedule of reinforcement in sessions comprised of five 20-min components. The dose of remifentanil per-ratio completed was increased during successive components of the session to determine dose-effect curves. A second group was trained under a comparable schedule with cocaine injections (0, 0.03-1.0 mg/kg/inj), and a third group with food reinforcement using 0 to 4 food pellets per ratio completed in the successive components. The TLR4 antagonist, (+)-naltrexone (3.2-56 mg/kg, sc), its peripherally acting analog, (+)-N-methylnaltrexone (32-100 mg/kg, sc), or vehicle were administered before selected daily sessions. Control response rates were an inverted U-shaped function of remifentanil or cocaine dose or amount of food, with maxima at 1.0 µg/kg/inj, 0.32 mg/kg/inj, or 2 pellets/presentation, respectively. (+)-Naltrexone dose-dependently decreased the self-administration of remifentanil, cocaine, and rates of responding maintained by food presentation. In contrast, (+)-N-methylnaltrexone, at doses greater than active doses of (+)-naltrexone, did not significantly alter remifentanil self administration. The potency of (+)-naltrexone in decreasing behavior was slightly greater with cocaine self administration than with responding maintained by either remifentanil or food reinforcement, for which there was no difference in potency. Overall the present study suggests that the (+)-naltrexone produced decreases in self-administration of remifentanil and cocaine are centrally mediated though generalized to behaviors maintained by various reinforcers. Supported by NIDA IRP (JLK, BRS, KCR) and a DoD Contract PR110146 (LRW).

Disclosures: **C. Zanettini:** None. **T. Hiranita:** None. **L.R. Watkins:** None. **B.R. Selfridge:** None. **K.C. Rice:** None. **J.L. Katz:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.03/L2

Topic: C.17. Drugs of Abuse and Addiction

Support: PA DOH Tobacco CURE Funds SAP 4100055576

Title: Reversal of the sleep/wake cycle in heroin self-administering rats

Authors: ***A. A. COFFEY**, Z. GUAN, P. S. GRIGSON, J. FANG;
Penn State Col. of Med., Hershey, PA

Abstract: Addiction is a chronic, relapsing disease. In the United States, heroin abuse is increasing dramatically. Drug abuse and sleep disturbances interact to form a vicious cycle where drug abuse disrupts sleep, and sleep deficits spur on drug abuse. Rodents have been shown to entrain their activity to the availability of cocaine or methamphetamine during the light cycle, but there have been no assessments of sleep patterns. Alcoholism has been linked to an inverted circadian rhythm of melatonin secretion, providing some evidence for circadian disruptions in humans. With opioid abuse, a decrease in rapid eye movement (REM) sleep during withdrawal is the most commonly cited sleep deficit. Here, we used electroencephalography (EEG) and electromyography (EMG) to measure sleep patterns in male Sprague-Dawley rats over 20 days of acquisition of heroin self-administration, 14 days of abstinence, and a single day of extinction and reinstatement. The results showed that rats entrain their sleep/wake patterns to heroin availability during the light cycle as evidenced by a complete inversion of sleep/wake patterns compared to the saline controls. Over the first 36 hours of abstinence, the circadian patterns of wakefulness and NREM sleep are lost in heroin self-administering rats, after which the patterns re-entrain to the light/dark cycle. In addition, individual differences were evident whereby a reduction in REM sleep was found during the first three days of abstinence for high vs low drug takers. Ultimately, characterization of the drug-induced dysregulation of sleep and circadian rhythm may reveal new avenues for treatment.

Disclosures: **A.A. Coffey:** None. **Z. Guan:** None. **P.S. Grigson:** None. **J. Fang:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.04/L3

Topic: C.17. Drugs of Abuse and Addiction

Title: Attenuating reinstatement of drug-seeking using selective kappa opioid receptor antagonists

Authors: *E. R. DUNN-SIMS[1], C. TYSZKIEWICZ[1], A. SAWANT-BASAK[2], J. HEDDE[2], Z. HUGHES[2], A. N. MEAD[1];
[1]Pfizer Inc, Groton, CT; [2]Neurosci. Res. Unit, Pfizer Inc, Cambridge, MA

Abstract: Dysregulation of the brain-reward system has been associated with numerous psychiatric and neurological disorders and co-morbidity exists between mood disorders and addiction. Exposure to stress is associated with drug addiction in humans and can induce relapse and craving. Similarly, a variety of stressful stimuli can reinstate drug-seeking in animal models. Converging evidence have demonstrated that the kappa-opioid receptor (KOR) pathway plays a critical role in regulating dopamine release in areas of the brain highly associated with reward-related learning and are implicated in reward, stress and mechanisms underlying stimulus-controlled drug-seeking behavior. The objective of these studies was to investigate the dynorphin-KOR system and its involvement in stress-induced drug relapse using the novel selective KOR-antagonist, PF-04455242, and the prototype KOR-antagonist LY-2456302 in a reinstatement model of drug-seeking. Male Sprague-Dawley rats were initially trained to self-administer nicotine or fentanyl, I.V., under a fixed ratio schedule of reinforcement using a standard 2-lever choice design, with infusion-paired cues. Following acquisition, an extinction period commenced to dissociate the act of lever pressing from delivery of the drug. Reinstatement tests were then conducted using a within-subjects design, with reinstatement induced by the pharmacological stressor, yohimbine administered alone and in combination with drug-paired (nicotine or fentanyl) cues. For reinstatement tests, animals were treated with PF-04455242 or LY-2456302, S.C., prior to each session. Congruent with the literature implicating KORs with the rewarding/reinforcing effects of drugs, and specifically the association between relapse to drugs of abuse and exposure to stress-inducing stimuli, the present findings suggest KOR-antagonists attenuate reinstatement of drug-seeking in rats and this effect appears specific to seeking induced by stress. The effect of these antagonists in blocking reinstatement of drug-seeking behavior was compared to their affinity to bind to, and occupy, the KOR. Taken together, these observations support the potential benefit of KOR-antagonism in relapse prevention.

Disclosures: E.R. Dunn-Sims: None. **C. Tyszkiewicz:** None. **A. Sawant-Basak:** None. **J. Hedde:** None. **Z. Hughes:** None. **A.N. Mead:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.05/L4

Topic: C.17. Drugs of Abuse and Addiction

Title: The neurobiological effects of acute oxycodone exposure: Data from BOLD, diffusion tensor, and manganese imaging in rats

Authors: *C. F. FERRIS, K. MOORE, W. KENKEL, D. MARINI, P. KULKARNI; Psychology, Northeastern University, Ctr. for Translational NeuroImaging, Boston, MA

Abstract: Oxycodone (OXY) is used clinically to treat severe acute or chronic pain; however, when inappropriately prescribed or misused can lead to severe addiction. Indeed, just a short exposure to OXY can lead to drug dependence. Consequently, studies were undertaken to examine the immediate effects of this opioid agonist on brain activity to a single exposure and to four consecutive days of drug given once daily (2.5 mg/kg). We were interested in two specific questions: 1) What are the brain areas activated by peripherally administered OXY? This was addressed with BOLD imaging in awake rats in response to a single dose of OXY and brain mapping with manganese (Mn) in response to four consecutive days of drug exposure. 2) Are there structural changes in the brain with OXY exposure? This was addressed by DTI and quantitative anisotropy. All studies used a 3D MRI atlas of the rat brain segmented and annotated into 171 brain areas. When combined with computation analysis it was possible to reconstruct OXY-driven integrated neural circuits for each of the imaging protocols. Sprague Dawley rats were acclimated to the imaging protocol before data collection. Experiments were conducted using a Bruker Biospec 7.0T magnet. Functional BOLD images were acquired in awake rats given saline or OXY IP during a 25 min imaging session. Manganese imaging was performed before and after four days of OXY administration. Diffusion tensor MRI data were acquired for indices of anisotropy (IA) for use in a novel method of analysis to detect changes in gray matter microarchitecture. IA values from over 20,000 voxels were registered into the MRI atlas to identify difference between control and drug exposed rats. Within 15 min of OXY injection there were significant changes in BOLD signal. Positive BOLD signal was localized primarily to the amygdala and thalamus, while negative BOLD was primarily associated with the olfactory system and hypothalamus. Four days of consecutive OXY showed enhanced Mn contrast primarily in the amygdala. Changes in IA values were most prominent in the olfactory system, hypothalamus and amygdala. Oxycodone when given to healthy adult male rats causes robust BOLD signal changes from olfactory bulbs to brainstem. The amygdala was particularly interesting because it showed long-term activation with repeated oxycodone and alterations in microarchitecture. Also of interest was the absence of activity in the pain neural circuit and only modest activity in the dopaminergic system, areas associated with analgesia and reinforcement, respectively. Molecular and cellular studies are underway to identify the microarchitectural changes identified with quantitative anisotropy.

Disclosures: **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging, Animal Imaging Research. **K. Moore:** None. **W. Kenkel:** None. **D. Marini:** None. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.06/L5

Topic: C.17. Drugs of Abuse and Addiction

Title: Comparison of the psychostimulatory effect induced by methadone, buprenorphine and morphine

Authors: ***J. M. ANDERSEN**, S. KABASHI, I. BERGSETEREN, J. MØRLAND; Norwegian Inst. of Publ. Hlth., Oslo, Norway

Abstract: Methadone and buprenorphine are synthetic opioid drugs used to treat heroin addiction because they effectively reduce consumption of illegal heroin and facilitates retention in treatment. However, replacing heroin with another opioid may be perturbing since the new drug also might be addictive and have harmful effects related to drug dependence. Behavioral sensitization seen following repeated exposure to a specific drug may be central in the development of drug addiction and involves changes in brain striatal dopamine transmission. Surprisingly, in methadone or buprenorphine therapy this aspect has received little attention both in human and animal studies. In this work, locomotor activity (used as a measure of a psychostimulatory effect), brain pharmacokinetics and western blot analysis of DARPP-32 in striatum, nucleus accumbens, frontal cortex and hippocampus were investigated in mice exposed (s.c.) to different doses of methadone (12.5-100 umol/kg), buprenorphine (0.25-2 umol/kg) or morphine (15- 60 umol/kg) either acutely or for prolonged periods (5-15 days). Methadone and morphine stimulated locomotor activity dose-dependently, while the effect of buprenorphine was less prominent. After repeated exposure a strong sensitization of the activity was seen. However, the pharmacokinetics of the drugs remained unaffected. DARPP-32 was expressed in all the brain areas investigated, but phosphorylation of this protein was only seen in nucleus accumbens and striatum, areas which are directly related to drug addiction. No clear relationship between the locomotor activity and pDARPP-32 was found. In this study, the psychostimulatory effect of methadone and morphine was more similar than the effect of buprenorphine. This may be related to the fact that methadone and morphine are agonists while buprenorphine acts a partial agonist on the mu receptor, suggesting a possible difference in the cellular responses triggered by these molecules.

Disclosures: **J.M. Andersen:** None. **S. Kabashi:** None. **I. Bergseteren:** None. **J. Mørland:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.07/L6

Topic: C.17. Drugs of Abuse and Addiction

Support: Kopf Family Postdoctoral Fellowship

NIH R01DA029147

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Activation of calmodulin-dependent kinase II protein in the hippocampus of oxycodone self-administered adult C57Bl/6 mice

Authors: *D. P. SIMON, Y. ZHANG, M. KREEK;
Lab. of Biol. of Addictive Dis., Rockefeller Univ., New York, NY

Abstract: An epidemic of prescription opioid and heroin addiction is occurring in the United States, characterized by: 1) wide-spread abuse amongst all age groups, but with especially high numbers amongst adolescents 2) frequent morbidity and mortality due to overdose and 3) a high rate of relapse and long-term persistence of drug addiction. The surge in abuse of opioids highlights the necessity for a greater understanding of this disease. An often underappreciated aspect of addictions is the role of learning and memory. For example, the hedonic aspects of the drug become associated with the act of drug taking. Also, neutral environments/stimuli that become associated with drug use can trigger drug craving_consolidation and retrieval of contextual memories. Indeed, it has been hypothesized that formation and persistence of contextual memories facilitates compulsive drug taking. The activation of calmodulin dependent kinase II (CamKII) in the hippocampus has been shown to be a critical molecular mechanism in memory formation. Furthermore, numerous drugs of abuse including the opioids, have been shown to require CamKII activity for maintenance of drug-taking. To examine the function of CamKII activation in the hippocampus during oxycodone-reward learning, we conducted a self-administration experiment. Adult C57Bl/6 male mice were implanted with an intravenous catheter and allowed to self-administer oxycodone or saline 2 hours/day for 14 days (0.25mg/nose poke). As a critical control, whenever an animal self-administered oxycodone, another animal passively received an infusion of oxycodone (oxycodone-yoked controls). Mice were then sacrificed and total hippocampal protein lysate was analyzed by Western blot. We found a statistically significant increase in the amount of phosphorylated-CamKIIα protein in the hippocampus of oxycodone-self administered mice when compared to the oxycodone-yoked or

saline controls, though no difference in phosphorylated CamKIIβ or total CamKII protein of either isoform was detected. Furthermore, mice that received investigator-administered oxycodone for 10 days (3mg/kg/day) did not exhibit any change in total or phosphorylated CamKII protein, as determined by Western blot analysis. Taken together, these data strongly suggest that activation of CamKIIα plays a critical role in opioid addiction-like settings specifically involving learning and memory, but not in the primary hedonic effects of the drug itself. Improved knowledge of hippocampal function in opioid addiction may help lead to the development of medications for either prevention and/or treatment of opioid addiction.

Disclosures: D.P. Simon: None. **Y. Zhang:** None. **M. Kreek:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.08/L7

Topic: C.17. Drugs of Abuse and Addiction

Support: UMAC FHS Grant SRG2013-0003

UMAC FHS Grant MYRG2014-00093-FHS

Macau FDCT Grant 026/2014/A1

Title: Use of functional near-infrared spectroscopy to identify resting-state functional connectivity in heroin addicts

Authors: *H. IEONG[1,2], F. LU[1], X. LIN[1], Z. YUAN[1];
[1]Fac. of Hlth. Sci., Univ. of Macau, Macau Sar, Macao; [2]Inst. of Chinese Med. Sci., Univ. of Macau, Macau SAR, Macao

Abstract: Addiction is defined as a loss of control of substance use despite adverse consequences. In addition to intensive work done by the public health and social welfare, recent neuroimaging studies have revealed new findings on the abnormal functional connectivity in addicted brains during cognitive and emotional processing. However, many of the studies did not focus on the brains at rest. Moreover, not only did different drugs yield different findings, but also the relapse rate and treatment outcomes have not yet been significantly improved in the past 50 years. As such, a more comprehensive experimental approach such as examining the resting-state functional connectivity (rsFC) is thus needed to resolve this issue. In particular, it is widely recognized that dynamic rsFC strength could provide dramatic insights to identify and predict various psychiatric trajectories. In this study, cost-effective and non-invasive functional near-infrared spectroscopy (fNIRS) technique was utilized to capture and characterize the brain activity, then the dynamic rsFC was constructed based on the fNIRS recordings from 5 heroin

users and 6 normal control subjects in Macau. Our results provided novel information on the change of functional connectivity during resting-state in heroin abusers, particular in orbitofrontal cortex (OFC) and dorsolateral prefrontal cortex (DL-PFC). Our robust findings suggest that fNIRS is a valid neuroimaging tool for cognitive studies in establishing psychiatric profile in addiction.

Disclosures: H. Ieong: None. **F. Lu:** None. **X. Lin:** None. **Z. Yuan:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.09/L8

Topic: C.17. Drugs of Abuse and Addiction

Support: Genomic Seed Grand

Title: Differential effects of oxycodone, hydrocodone, and morphine on gene expression

Authors: *M. A. EMERY, M. BATES, P. J. WELLMAN, S. EITAN;
Psychology, Texas A&M Univ., College Station, TX

Abstract: Opioids (a term that refers to both opiates, the natural products obtained from the opium poppy, and synthetic opioids) are the most commonly used drugs for managing moderate-to-severe pain. However, their chronic use is complicated by the development of tolerance, hyperalgesia, dependence, reward, and abuse. Exposure to opioid analgesics has been associated with altered responses of D2-like dopamine receptors (D2DRs). Disturbances in the responses of the D2DRs would be expected to have important implications for the abuse potential of opioids, other drugs of abuse, and alcohol. Moreover, the D2DRs were also suggested to be involved in the pathophysiology of a wide range of affective and psychotic disorders. Our previous studies demonstrated that various opioids modulate the responses of D2DRs in differential degrees. Specifically, oxycodone, hydrocodone, and morphine differentially modulate baseline activation levels of signaling molecules. This in turn results in ligand-selective effects on both the signaling and behavioral responses to a D2/D3 dopamine receptor agonist. Mice pretreated with oxycodone showed significantly greater locomotor supersensitivity to quinpirole than did mice pretreated with equianalgesic doses of morphine, while mice pretreated with equianalgesic doses of hydrocodone showed sensitivity between that of mice treated with morphine and oxycodone. Additionally, morphine, oxycodone, and hydrocodone have differential efficacies in relieving burn pain and minimizing the development of chronic pain. These studies demonstrated a complex interplay between opioid receptors and D2DRs, and support the notion that various opioids carry differential risks to the dopamine reward system. Thus, in the current study we examined the differential effect of oxycodone, hydrocodone, and morphine on modulation of

striatal gene expression. This information highlights differences between opioids in modulating the responses of the reward system and should be considered when prescribing opioid pain medication, in order to balance effectiveness with minimal risk.

Disclosures: **M.A. Emery:** None. **M. Bates:** None. **P.J. Wellman:** A. Employment/Salary (full or part-time):; Texas A&M Univ. **S. Eitan:** A. Employment/Salary (full or part-time):; Texas A&M Univ.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.10/L9

Topic: C.17. Drugs of Abuse and Addiction

Support: MOST 103-2314-B-037 -077 -MY2

Title: Long term opioid use induces systemic inflammation and down regulation of TGF-β1 and BDNF

Authors: ***S.-L. CHEN**[1], R.-B. LU[2], L.-E. YU[1];
[1]Kaohsiung Med. Univ., Kaohsiung, Taiwan; [2]Dept. of Psychiatry, Col. of Med. & Hospital, Natl. Cheng-Kung Univ., Tainan, Taiwan

Abstract: Objective: The over activation of inflammatory cytokine and dysfunction of the neurotrophic system might be related to the development of opioid dependence. Recent studies show that proinflammatory cytokines might be related to the development of opioid dependence (physiological, psychological, or both). In addition, brain-derived neurotrophic factor (BDNF) may be important in synaptic plasticity and neuron survival, and may become a key target in the physiopathology of long-term opioid use. Thus, we investigated the expression of plasma cytokines and BDNF concentrations in heroin-dependent patients. Furthermore, the transforming growth factor (TGF)-β1, a key immunoregulatory factor for central nervous system was also evaluated. Methods: The pretreatment expression levels of plasma cytokines, TGF-β1 and BDNF levels in 142 heroin-dependent male patients and 75 male healthy controls were evaluated by the enzyme-linked immunosorbent assay. Results: Plasma TNF-α, Interleukin (IL)-6 (IL-6), IL-8 and CRP were significantly higher in patients with long term heroin used. However, plasma TGF-β1 and BDNF levels were significantly lower in patient with long term heroin used. The plasma BDNF levels were significantly positive correlated to the TGF-β1 levels and negative correlated to the IL-6 levels. Conclusion: Long term used heroin induced systemic inflammation and neurotrophic factor down regulation. Furthermore, plasma BDNF might modulate the inflammatory factors (TGF-β1 and IL-6) concentration. The systemic inflammation, downregulation of TGF-β1 and BDNF might caused the neuronal inflammation and less

neuronal protective effects after long term opioid used. Providing the anti-inflammatory and neuronal protective agents might benefit to the treatment of opioid dependence.

Disclosures: **S. Chen:** None. **R. Lu:** None. **L. Yu:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.11/L10

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA031442-03

Title: Morphine modulates mouse hippocampal progenitor cell lineages via PKCε-dependent ERK activation and TRBP phosphorylation

Authors: **C. XU**[1], H. ZHENG[2], H. H. LOH[1], *P.-Y. LAW[1];
[1]Univ. of Minnesota, Minneapolis, MN; [2]South China Inst. for Stem Cell Biol. and Regenerative Med., Guangzhou, China

Abstract: Morphine regulates adult neurogenesis by modulating miR-181a maturation and subsequent hippocampal neural progenitor cell (NPC) lineages. By using NPCs cultured from PKCε or β-arrestin2 knockout mice and the MEK inhibitor U0126, we demonstrate that regulation of NPC differentiation via the miR-181a/Prox1/Notch1 pathway exhibits ligand-dependent selectivity. In NPCs, morphine and fentanyl activate ERK via the PKCε- and β-arrestin-dependent pathways, respectively. After fentanyl exposure, the activated phospho-ERK translocates to the nucleus. Conversely, after morphine treatment phospho-ERK remains in the cytosol and is capable of phosphorylating TRBP, a cofactor of Dicer. This augments Dicer activity and promotes the maturation of miR-181a. Furthermore, by using NPCs transfected with wild type TRBP, SΔA and SΔD TRBP mutants, we confirmed the crucial role of TRBP phosphorylation in Dicer activity, miR-181a maturation, and finally the morphine-induced astrocyte-preferential differentiation of NPCs. Thus, morphine modulates the lineage-specific differentiation of NPCs by PKCε-dependent ERK activation with subsequent TRBP phosphorylation and miR-181a maturation.

Disclosures: **C. Xu:** A. Employment/Salary (full or part-time):; University of Minnesota. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIDA DA031442-03. **H. Zheng:** None. **H.H. Loh:** None. **P. Law:** A. Employment/Salary (full or part-time):; University of Minnesota. B. Contracted Research/Research Grant (principal investigator for a drug study,

collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIDA DA031442-03.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.12/L11

Topic: C.17. Drugs of Abuse and Addiction

Support: USU grant R088305315

Title: Individual differences in morphine-induced antinociception and intravenous morphine self-administration in rats

Authors: *K. CHOI[1], K. NISHIDA[2], R. J. URSANO[2];
[1]Psychiatry, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]Psychiatry, Uniformed Services Univ., Bethesda, MD

Abstract: As many as one hundred million people may suffer from chronic pain and over two million Americans abuse opioid pain medications. Although morphine is a highly potent opioid analgesic used for pain management, its analgesic efficacy fades gradually during repeated administration. Thus, progressively higher doses of morphine are required to achieve comparable analgesic effects. Previous studies with rodents have reported that experimenter-administered repeated morphine induces tolerance to analgesic effects and morphine withdrawal increases pain sensitivity (hyperalgesia). However, it is not clear whether intravenous morphine self-administration also induces tolerance and hyperalgesia in rodents. Male Sprague-Dawley rats self-administered intravenous morphine (0.5 mg/kg/infusion) for three weeks (4 hrs per day, 5 days per week). Using a noxious (thermal) behavioral paradigm, antinociceptive effects of morphine, tolerance to repeated morphine and withdrawal-induced hyperalgesia were measured in morphine self-administered animals. On day 1, morphine self-administered animals showed antinociception to thermal pain and individual differences in antinociception. However, on day 3, morphine self-administered animals started to develop tolerance to antinociceptive effects of morphine. Spontaneous withdrawal from chronic morphine self-administration (MSA) induced hyperalgesia in these animals. Individual differences in initial morphine-induced antinociception were correlated with development of addiction-like behavior following chronic MSA. Our findings support the results from previous studies that used morphine injections and further suggest individual differences in analgesic responses and vulnerability to develop opioid addiction. This observation is important because poor responders to opioid medications require higher doses of opioids and other pain medications, which may increase probability of substance abuse in vulnerable individuals. Early identification and intervention of vulnerable individuals may improve the treatment strategy for pain management and opioid abuse.

Disclosures: **K. Choi:** None. **K. Nishida:** None. **R.J. Ursano:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.13/L12

Topic: C.17. Drugs of Abuse and Addiction

Support: NSC 101-2320-B-182-040-MY3

Chang Gung Memorial Hospital CMRPD1C0522

Healthy Aging Research Center EMRPD1E1641

Title: Altered nociception and morphine tolerance in neuropeptide FF receptor type 2 over-expressing mice

Authors: ***Y.-T. LIN**[1], S.-C. KAO[3], Y.-J. DAY[3], C.-C. CHANG[5], J.-C. CHEN[1,2,4];
[1]Grad. Inst. of Biomed. Sci., [2]Healthy Aging Res. Ctr., Chang Gung Univ., Tao-Yuan, Taiwan;
[3]Dept. of Anesthesiol., [4]Neurosci. Res. Ctr., Chang Gung Mem. Hosp., Tao-Yuan, Taiwan;
[5]Dept. of Chem., Fu Jen Catholic Univ., New Taipei City, Taiwan

Abstract: The neuropeptide FF system is thought to act as an anti-opioid modulator and plays a role in nociception, morphine antinociception and dependence. Two receptor subtypes, NPFFR1 and NPFFR2, have been identified, but their respective roles in these processes remain uncertain. In the present study, the role of NPFFR2 was investigated using transgenic mice over-expressing NPFFR2 in addition to a selective NPFFR2 agonist AC-263093. NPFFR2 Tg mice exhibited increased sensitivity to both mechanical and thermal noxious stimuli compared to the WT mice, while the antinociceptive effects of morphine at three different doses (6.25, 12.5, and 25 mg/kg, s.c.) were similar in both strains. The development of tolerance to morphine antinociception after chronic morphine treatment (12.5 mg/kg, s.c.; twice daily × 5 days) was attenuated in NPFFR2 Tg mice when compared to WT mice. Similarly, WT mice receiving AC-263093 pretreatment (2.5 mg/kg, i.p.) showed attenuated morphine tolerance compared to vehicle controls. Most naloxone-precipitated morphine withdrawal symptoms were not attenuated in NPFFR2 Tg mice, with the exception that wet dog shake was significantly reduced. Both NPFFR2 Tg and WT mice displayed similar degree of morphine rewarding. Our results suggest that NPFFR2 is mainly involved in the modulation of nociception and tolerance to morphine antinociception.

Disclosures: **Y. Lin:** None. **S. Kao:** None. **Y. Day:** None. **C. Chang:** None. **J. Chen:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.14/L13

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA025674

NIH Grant DA034886

Title: Modeling prenatal and postnatal oxycodone exposure using self-administration

Authors: F. M. VASSOLER, A. KUBEREK, C. WYSE, *E. M. BYRNES;
Tufts Univ. Cummings Sch. Vet Med., North Grafton, MA

Abstract: The abuse of opiates has emerged as a major public health issue. Of growing concern are the numbers of women abusing opiates during pregnancy, with a significant increase observed over the past decade. A large number of these women are using prescription opiates containing oxycodone. While neonatal abstinence syndrome is one consequence of prenatal opiate use, the long-term consequences associated with use have not been well-documented. Additionally, animal models of prenatal oxycodone exposure are lacking. To address this gap, we have established a prenatal oxycodone self-administration model in rats. The goal of these studies are twofold; 1) develop a model with high face validity that can be used to make informed predictions about potential long-term neurodevelopmental effects in offspring; and 2) utilize this model to better understand variations in female use patterns that may be a natural function of the reproductive state and environmental conditions. In the current set of studies cycling female Sprague-Dawley (200-225g) rats were fitted with indwelling jugular catheters. One week later females began daily 1h self-administration sessions in standard self-administration chambers (Med Associates) with one lever press resulting in one infusion of oxycodone (0.1 mg/kg/infusion). During this period, females were assessed daily for stage of estrous cycle. Following at least three weeks of daily sessions, proestrus females were placed overnight with breeder males. Pregnancy was confirmed by the presence of sperm in the vaginal lavage. Self-administration sessions continued throughout pregnancy and during the postpartum period with doses adjusted based on increasing and decreasing bodyweight. On postnatal day 1 (PND1), half of the subjects had their pups removed while the other half remained with their pups. Additionally on PND1, brains were harvested from one male and one female offspring from each litter. Data indicate that levels of responding for oxycodone increase during pregnancy and even more so during the postpartum period. Preliminary findings suggest that the presence or absence of pups influences the level of responding. Gene expression studies are ongoing in PND1 brains as are studies examining the effects of prenatal oxycodone on maternal behavior and pup ultrasonic vocalizations. These findings represent the initial phase of model development in the area of prenatal opiate abuse.

Disclosures: **F.M. Vassoler:** None. **A. Kuberek:** None. **C. Wyse:** None. **E.M. Byrnes:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.15/L14

Topic: C.17. Drugs of Abuse and Addiction

Title: A wireless electrophysiology system designed for the preclinical development of translatable biomarkers for treatment of substance use disorders

Authors: *J. K. DASILVA[1], S. KREUSER[3], T. CHAPPIE[2], P. TRAPA[4], A. N. MEAD[1], D. P. NGUYEN[4];
[1]Global Safety Pharmacology, Drug Safety Res. and Develop., Pfizer, Inc., Groton, CT; [2]Neurosci. Med. Chem., Pfizer, Inc., Cambridge, MA; [3]Worldwide Comparative Med., Pfizer, Inc, Groton, CT; [4]Translational Modeling and Simulation, Pharmacodynamics and Metabolism, Pfizer, Inc, Cambridge, MA

Abstract: Drug addiction is a widespread disorder with a large unmet need for treatment options. In the process of developing therapies, two major goals have been: 1) to understand how a potential therapy reduces the motivation to seek and take a drug, and 2) to develop a non-invasive biomarker that would be predictive of therapeutic efficacy in the clinic. The reinstatement model is a rodent model of drug-seeking and relapse, in which conditioned reinforcement drives relapse-like behavior, and has been used to investigate the efficacious properties of potential treatments for substance use disorders. In addition, event-related potentials (ERPs) have been shown to correlate with specific neural coding pathways in rodent models of decision making and attention, and thus when combined with the reinstatement model, provides a powerful tool for understanding how pharmacologically-based neural circuit modulation correlates with reduced drug seeking behavior. Simultaneously, the dose-response of ERP modulation may provide a non-invasive clinical endpoint for assessing an early sign of efficacy in the clinic. In order to achieve ERP recordings in the drug discovery setting, we designed a single system that could overcome a number of design constraints: 1) wireless recording to prevent interference with drug delivery catheters, 2) scalable to a large number of concurrently tested animals, 3) daily experiments are quick and easy to conduct, 4) easily retrofits with existing behavioral setups, and 5) has the temporal precision to resolve millisecond level features in the neuroelectrophysiology data. We demonstrate that we were able to observe, with high precision, ERPs that are driven by drug-associated cues, and observed clear alterations in ERP N1-P2 complex latency over the course of the reinstatement session, which may reflect real-time alteration in the motivation to self-administer a drug. Finally, we outline a translational strategy based on these ERP data in order to clearly demonstrate how these ERPs may inform and bridge, preclinical and clinical strategies.

Disclosures: **J.K. DaSilva:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **S. Kreuser:** None. **T. Chappie:** None. **P. Trapa:** None. **A.N. Mead:** None. **D.P. Nguyen:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.16/L15

Topic: C.17. Drugs of Abuse and Addiction

Support: DA09082

Title: Stress-induced activation of amygdalar corticotropin releasing factor neurons projecting to the locus coeruleus in morphine dependent rats

Authors: ***B. A. REYES**[1], N. HELDT[1], M. HENRY[2], G. DROLET[2], E. J. VAN BOCKSTAELE[1];
[1]Pharmacol. & Physiol., Drexel Univ., Philadelphia, PA; [2]Neurosci., Univ. Laval, Ctr. de Recherche du CHU de Quebec, Quebec, QC, QC, Canada

Abstract: Chronic opiate exposure promotes the development of long-term adverse consequences including tolerance and physical dependence as a result of persistent alterations in brain neurons. The locus coeruleus-norepinephrine (LC-NE) system is implicated in arousal and cognition associated with the stress response. Previous electrophysiological studies have demonstrated that the LC-NE is sensitized to corticotropin-releasing factor (CRF) following chronic morphine exposure. While it is known that the LC receives CRF afferents from multiple brain regions including the central nucleus of the amygdala (CeA), the mechanism underlying enhanced sensitivity to stress following chronic opiate use remains unknown. In a first study, male Sprague-Dawley rats received morphine via subcutaneous delivery of pellets. Controls received placebo pellets. *In situ* hybridization labeling of CRF mRNA in the amygdala revealed a lack of statistical significance in expression levels across groups. To further characterize stress-induced neuronal activation of LC-projecting amygdalar CRF neurons under conditions of morphine dependence, male rats received microinjections of fluorescent latex microspheres (Retrobeads) into the LC. Five days later, rats were implanted with morphine or placebo pellets. Seven days post-implantation, a subset of rats was exposed to a single 15-minute swim. Cell counts revealed that c-Fos expression in the CeA was significantly increased in the swim stress groups irrespective of morphine exposure ($P < 0.05$). Morphine treatment further increased c-Fos expression ($P < 0.05$) when compared to placebo irrespective of stress exposure, and compared to morphine alone. Interestingly, more than half of the c-Fos labeled neurons in the morphine dependent stress-exposed group were observed in CRF amygdalar neurons that projected to the LC. These results indicate that morphine exposure increases CRF amygdalar responses to stress and that this increased activation impacts the LC. Such cellular adaptations have important

consequences for increasing brain noradrenergic tone and may predispose chronic opiate users to hyper-arousal, a symptom observed in many stress-related psychiatric disorders.

Disclosures: **B.A. Reyes:** None. **N. Heldt:** None. **M. Henry:** None. **G. Drolet:** None. **E.J. Van Bockstaele:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.17/L16

Topic: C.17. Drugs of Abuse and Addiction

Support: National Science Foundation of China Grant 81171257

Title: Acute combined low/high burst stimulation of the lateral habenula attenuates cue-induced heroin seeking in rats

Authors: *F. ZHANG;
Ningbo Addiction Res. and Treatment Ctr., Zhejiang, China

Abstract: The lateral habenula (LHb) is critical for the modulation of rewarding process and drug addiction. The aim of the present study is to observe the effects of deep-brain-stimulation (DBS) in the LHb on heroin seeking behavior. An electrode was implanted in the LHb and rats were trained to establish stable heroin self-administration behavior (0.05 mg/kg/infusion) for daily 4h session under a FR1 schedule. Results showed that pretreatment with high- frequency (100Hz, 0.5ms, 0.2mA) or a combined low/high frequency (alternating between 10Hz and 100Hz, 0.5ms, 0.2mA) stimulation of the LHb for 15min could significantly enhance heroin self-administration, whereas no effect was observed with low-frequency (10Hz, 0.5ms, 0.2mA) stimulation. Cue-induced heroin seeking was tested after extinction of heroin self-administration for 14 days. After extinction, cue-induced reinstatement of heroin seeking could be attenuated only by acute pretreatment with the combined DBS. Microdialysis showed that dopamine level in the nucleus accumbens (NAc) could be decreased after low-frequency, while increased after high or combined DBS in the LHb. We postulate that the effect of combined DBS in LHB on heroin seeking may be via modulation of the neuronal activity of the ventral tegmental area and the NAc dopamine projections, suggesting the LHb might be a promising treatment target for drug addiction treatment.

Disclosures: **F. Zhang:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.18/L17

Topic: C.17. Drugs of Abuse and Addiction

Support: IRP/NIDA/NIH

Title: Role of projections from ventral subiculum to nucleus accumbens shell and ventral medial prefrontal cortex in context-induced reinstatement of heroin seeking

Authors: *J. M. BOSSERT[1], S. ADHIKARY[1], R. M. ST. LAURENT[1], N. J. MARCHANT[1,3], H. L. WANG[2], M. MORALES[2], Y. SHAHAM[1];
[1]Behavioral Neurosci., [2]Integrative Neurosci., NIH, NIDA, IRP, Baltimore, MD; [3]Florey Inst. of Neurosci. & Mental Health, Univ. of Melbourne, Parkville, Australia

Abstract: Background: In humans, exposure to contexts previously associated with heroin use can provoke relapse. In rats, exposure to heroin-paired contexts after extinction of drug-reinforced responding in a different context reinstates heroin seeking. We previously demonstrated a causal role for projections from ventral medial prefrontal cortex (mPFC) to accumbens shell in context-induced reinstatement of heroin seeking. Because ventral subiculum sends glutamate projections to both accumbens shell and ventral mPFC, we sought to determine whether these projections also contribute to this reinstatement. Methods: We trained rats to self-administer heroin in a distinct context and then extinguished lever pressing in a non-drug-associated context; infusions or lever presses were paired with a discrete cue. We then tested the rats in the heroin- and/or extinction-associated contexts under extinction conditions. We first used the retrograde tracer Fluoro-Gold in combination with Fos to assess whether the ventral subiculum→accumbens shell or ventral subiculum→ventral mPFC pathway is activated during context-induced reinstatement of heroin seeking. We then employed an anatomical disconnection procedure to determine whether these projections are functionally involved in this reinstatement. Results: Exposure to the heroin context, but not the extinction context, reinstated lever pressing. Context-induced reinstatement was associated with increased Fos expression in ventral subiculum neurons, including those that project to accumbens shell or ventral mPFC. Contralateral and ipsilateral disconnection of ventral subiculum from accumbens shell decreased context-induced reinstatement. We are currently examining whether disconnection of ventral subiculum from ventral mPFC inhibits this reinstatement. Conclusions: Results suggest that glutamatergic projections from ventral subiculum to accumbens shell play a critical role in context-induced relapse to heroin seeking.

Disclosures: J.M. Bossert: None. **S. Adhikary:** None. **R.M. St. Laurent:** None. **N.J. Marchant:** None. **H.L. Wang:** None. **M. Morales:** None. **Y. Shaham:** None.

Poster

319. Opioids

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 319.19/L18

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01DA021421

NIH Grant U54DA038999

Title: Machine learning identifies distinct behavioral markers for opiate and stimulant addiction

Authors: *J. L. VASSILEVA[1], W.-Y. AHN[2], F. G. MOELLER[2];
[1]Dept. of Psychiatry, [2]Psychiatry, Virginia Commonwealth Univ., Richmond, VA

Abstract: The development of objective markers for addiction can innovate its prevention and treatment. Abundant evidence indicates that drug addiction is characterized by personality, psychiatric, and neurocognitive manifestations of impulsivity, one of the most viable endophenotypic markers for addiction. However, it is unclear which of its various dimensions would have the highest predictive utility for addiction and whether addiction to different classes of drugs would be characterized by different impulsivity profiles. To address these gaps, we used a machine learning approach, which holds promise for discovering predictive markers of disease. We conducted two machine-learning studies, using different indices of impulsivity as predictors of stimulant and opiate dependence. In Study 1, we recruited current stimulant (cocaine) users and healthy controls (HCs) in USA who completed self-reports of trait impulsivity and neurocognitive tasks of impulsive choice and impulsive action. A machine learning model was fitted on the training set using 5-fold cross validation and we tested its out-of-sample classification accuracy in the test set. The area under the curve (AUC) of the ROC curve was 0.90 in the test set. In Study 2, we tested HCs and individuals with past mono-dependence on heroin or amphetamine, currently in protracted abstinence. The study was done in Bulgaria where poly-substance dependence is still uncommon. Machine learning analyses revealed that the AUC of the ROC curve in the test set was 0.85 and 0.75 for the classification of past heroin and amphetamine dependence, respectively. Heroin and amphetamine dependence were predicted by distinct multivariate personality and neurocognitive impulsivity profiles. Delay discounting predicted stimulant dependence in both studies. Our results demonstrate the promise of machine-learning approaches to extract features predictive of group membership with high degree of accuracy and drug-class specificity and highlight how decision science and advanced statistical methods can inform clinical science. The results suggest that behavioral measures of impulsivity and decision-making can be objective markers of drug addiction. Results suggest that delay discounting may be a viable endophenotypic marker for stimulant (but not opiate) addiction. Our findings suggest that different mechanisms may underlie stimulant and opiate addiction, challenging the unitary account of drug addiction. This line of work may shed light on the development of affordable and easy-to-administer standardized tests that can be used to assess individuals' risk to addiction to different classes of drugs in clinical settings.

Disclosures: **J.L. Vassileva:** None. **W. Ahn:** None. **F.G. Moeller:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.01/L19

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA015835

NIDA Grant F32DA040414

Title: Examining protein synthesis in the nucleus accumbens after withdrawal from extended-access cocaine self-administration

Authors: ***M. T. STEFANIK**, M. MILOVANOVIC, M. E. WOLF;
Neurosci., Rosalind Franklin Univ. of Sci. and Medici, North Chicago, IL

Abstract: During withdrawal from extended-access cocaine self-administration, there is a progressive intensification (incubation) of cue-induced cocaine craving that is associated with numerous synaptic adaptations in the nucleus accumbens (NAc). Recent work from our lab suggests these adaptations are maintained by dysregulated local protein translation. Aberrant translation has a profound impact on cellular function and is a key feature in Fragile X syndrome and some other disorders of the nervous system. Treatments to normalize protein synthesis have proven successful in reversing some behavioral and cellular abnormalities in a mouse model of Fragile X. Currently, little is known about mechanisms regulating translation in the NAc. Furthermore, the possibility of long-term alterations in translation following cocaine exposure has been largely uninvestigated and provides an intriguing novel target for therapeutic intervention. We examined the hypothesis that incubation of cocaine craving is associated with dysregulation of protein translation in the NAc. Male Sprague Dawley rats underwent extended-access cocaine or saline self-administration (6hr/10days, 0.5mg/kg/infusion), followed by >40 days of withdrawal. We used 35S-Met/Cys incorporation to measure protein translation in NAc tissue. Preliminary data indicate that overall translation is not different between cocaine and saline groups, suggesting that translation of only a small subset of proteins may be differentially regulated. Work is underway to compare patterns of translation using bioorthogonal noncanonical amino acid tagging (BONCAT) of newly synthesized proteins to specifically examine translation rates of key synaptic targets either using immunoprecipitation or through an unbiased mass spectrometry approach. We are also comparing the regulation of translation in cocaine versus saline rats by mGluR and NMDA receptors. These studies are the first to characterize how synaptic transmission regulates protein translation in the NAc under basal

conditions and whether drugs of abuse cause persistent alterations in the synthesis of proteins linked to addiction.

Disclosures: **M.T. Stefanik:** None. **M. Milovanovic:** None. **M.E. Wolf:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.02/L20

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA034943

NIH Grant DA015835

NIH Grant DA009621

NIH Grant DA029099

NIH Grant DA36963

Title: Cocaine self-administration alters calcium signaling mediated by NMDA and AMPA receptors in dendritic spines of rat nucleus accumbens neurons

Authors: *D. T. CHRISTIAN, C. A. BRIGGS, M. E. WOLF, G. E. STUTZMANN; Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Cue-induced cocaine craving intensifies or incubates during withdrawal from extended-access cocaine self-administration. After prolonged withdrawal, incubated seeking is mediated by GluA2-lacking, Ca2+-permeable AMPARs (CP-AMPARs) that accumulate in the nucleus accumbens (NAc). The functional consequences of CP-AMPAR accumulation have been characterized using whole-cell patch-clamp recordings, but, to our knowledge, no studies have characterized Ca2+ entry through CP-AMPARs at the level of NAc dendritic spines following the incubation of cocaine craving. In addition to CP-AMPARs, NMDARs play a major role in Ca2+ signaling and have been increasingly implicated in cocaine-induced neuroadaptations. Here we measured Ca2+ entry into MSN dendritic spines in the rat NAc following >40 days of withdrawal from saline or cocaine self-administration. We utilized 2-photon microscopy, whole cell electrophysiology, and photo-uncaging of either MNI-L-Glutamate (in the presence of APV) to activate CP-AMPARs or MNI- NMDA to activate NMDARs. We found that cocaine rats exhibited an increase in the proportion of spines responding to MNI-Glutamate + APV, suggesting that some CP-AMPARs are added to spines that did not previously possess them. In addition, cocaine rats demonstrated a significant reduction in the percentage of spines responding to MNI-NMDA photolysis compared to saline

control rats. We are evaluating potential explanations for this effect. Support: DA034943 (G.E.S), DA015835, DA009621, DA029099 (M.E.W) and postdoctoral NRSA DA36963 (D.T.C.).

Disclosures: D.T. Christian: None. **C.A. Briggs:** None. **M.E. Wolf:** None. **G.E. Stutzmann:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.03/L21

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA015835

NIH Grant F31 DA036950

NIH Grant K99 DA038110

Title: Comparison of trafficking mechanisms of calcium-impermeable and calcium-permeable AMPA receptors in rat nucleus accumbens neurons co-cultured with prefrontal cortex neurons

Authors: *N. M. CHAUHAN[1], C. T. WERNER[1], C. H. MURRAY[1], J. M. REIMERS[2], J. A. LOWETH[1], M. E. WOLF[1];
[1]Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [2]Tacoma Community Col., Tacoma, WA

Abstract: Glutamatergic transmission in the nucleus accumbens (NAc) is critical for motivated behaviors, including drug addiction. In an animal model of cocaine addiction called the "incubation" model, rats exhibit progressive intensification or "incubation" of cue-induced cocaine craving during withdrawal from extended access cocaine self-administration. We have shown that this cocaine regimen leads to the accumulation of GluA2-lacking, Ca2+-permeable AMPA receptors (CP-AMPARs) in the NAc and that these high conductance AMPARs mediate the expression of incubated cue-induced cocaine craving after prolonged withdrawal (Conrad et al., 2008). To understand how this occurs, it would be helpful to know whether different mechanisms regulate the trafficking of CP-AMPARs versus the GluA2-containing, Ca2+-impermeable AMPA receptors (CI-AMPARs) that normally dominate synaptic transmission in the NAc. Because receptor trafficking is difficult to study *in vivo*, we are using a model system consisting of rat NAc neurons co-cultured with prefrontal cortex (PFC) neurons from enhanced cyan fluorescent protein (ECFP)-expressing mice. The cortical neurons restore excitatory input onto the NAc neurons but can be distinguished based on their fluorescence. NAc medium spiny neurons (MSN) in this co-culture system express high levels of CP-AMPARs, recapitulating the

state of the NAc after incubation of cocaine craving (Sun et al., 2008). The goal of this study is to compare the regulation of CP-AMPARs (homomeric GluA1) and CI-AMPARs (GluA1A2) with respect to: 1) rate of receptor internalization and receptor cycling (rate at which tagged surface receptors are replaced by untagged receptors), 2) effect of protein synthesis inhibition on surface receptor levels and cycling, and 3) effect of increasing or decreasing synaptic activity on surface receptor levels and their cycling. So far, our results indicate that: 1) GluA1 and GluA2 exhibit similar rates of constitutive internalization and cycling. 2) Brief protein synthesis inhibition reduces surface levels of both CI-AMPARs and CP-AMPARs. 3) Following activity blockade (CNQX, 24 h), CI-AMPARs, but not CP-AMPARs, show increased surface expression and cycling. In contrast, increased activity (bicuculline, 24 h) decreases both CI-AMPAR and CP-AMPAR surface expression. These studies will better our understanding of mechanisms that shape excitatory transmission in the NAc, which has translational implications related to drug addiction and relapse.

Disclosures: **N.M. Chauhan:** None. **C.T. Werner:** None. **C.H. Murray:** None. **J.M. Reimers:** None. **J.A. Loweth:** None. **M.E. Wolf:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.04/L22

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA015835 (M.E.W.)

Predoctoral NRSA F31DA036950 (C.T.W)

Postdoctoral NRSA DA040414 (M.T.S.)

Title: Regulation of protein translation following prolonged withdrawal from extended-access cocaine self-administration with or without cocaine memory retrieval

Authors: ***C. T. WERNER**, M. T. STEFANIK, M. MILOVANOVIC, M. E. WOLF; Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Vulnerability to relapse in cocaine addicts is often attributed to craving evoked by exposure to cues previously paired with the drug. Studies in animal models of addiction have shown that drug memories, upon retrieval, become labile and must undergo reconsolidation to be returned to long-term storage, a process that represents a potential therapeutic target for treating addiction. It is therefore important to understand mechanisms mediating memory retrieval and reconsolidation. It has been shown that de novo protein synthesis is necessary for retrieval and/or reconsolidation of cocaine memories. In addition, numerous studies have implicated mTOR-

related signaling, a pathway that regulates protein translation, in cocaine-dependent plasticity. Lastly, previous work from our lab has shown that ongoing protein translation is required to maintain cocaine-dependent synaptic adaptations in the nucleus accumbens (NAc during prolonged withdrawal. The goal of this study was to determine if the regulation of protein translation, and particular the mTOR pathway, is altered following an extended-access cocaine self-administration regimen that produces progressive intensification, or "incubation", of cue-induced craving. To study this, we collected NAc tissue from rats that self-administered saline or cocaine and underwent ~50 days of withdrawal. Half the rats in each group experienced a cue-induced seeking test. In our first experiment, we prepared a P2 fraction and evaluated a number of proteins involved in regulation of translation, including two factors involved in translation initiation and elongation, eIF2 and eEF2, respectively. We found retrieval-dependent but treatment-independent increases in phosphorylated and total eIF2 and phosphorylated but not total eEF2. In our second experiment, we prepared NAc synaptoneurosomes to better isolate postsynaptic processes. Analysis of eIF2, eEF2 and other translational regulators is underway.

Disclosures: C.T. Werner: None. **M.T. Stefanik:** None. **M. Milovanovic:** None. **M.E. Wolf:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.05/L23

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA019666

K02 DA035459

P30 NS062158

Breyer-Longden Family Research Fund

Title: Reinstatement to drug-seeking behavior following cocaine, cues, and stress results in synaptic depotentiation of glutamatergic synapses in the nucleus accumbens

Authors: *S. R. EBNER, M. C. HEARING, E. B. LARSON, M. J. THOMAS;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Relapse to drug-seeking behavior during abstinence, often precipitated by drug re-exposure, cues, or stress, is a major obstacle to long-lasting addiction recovery. Understanding the neurobiology of drug craving and relapse is likely to provide new means for addiction treatment. The nucleus accumbens (NAc), a key target for addictive drugs, is a critical interface of mesolimbic dopamine circuitry with excitatory cortical afferents in regulating motivation and

drug seeking. Plasticity of glutamatergic synapses in the NAc is induced by *in vivo* cocaine and appears to contribute to increased drug-seeking behavior. Repeated experimenter-administered cocaine potentiates synaptic strength in NAc medium spiny neurons (MSNs) following abstinence, while subsequent re-exposure to cocaine (temporarily) reverses this effect. The extent to which this pattern of plasticity occurs following self-administration of cocaine, and whether cue-, or stress-primed reinstatement elicits depotentiation is unknown. Using a combination of cocaine self-administration with NAc whole-cell recordings in an *ex vivo* brain slice preparation, we examined plasticity at glutamatergic synapses in NAc shell MSNs following reinstatement of drug-seeking behavior precipitated by saline, cocaine, stress, and drug-associated cues. Mice self-administered 0.5mg/kg/infusion cocaine for 10 days before 8-12 days of extinction training. To reinstate drug seeking, mice were exposed to a priming injection of saline, cocaine (10mg/kg), or yohimbine (2.5mg/kg) prior to, or drug-associated cues during reinstatement testing. As predicted, cocaine-, yohimbine-, and cue-primed animals exhibited greater reinstatement behavior than saline injected animals (228.2±55.9%, 268.0±42.8%, 169.0±21.0%, 95.6±10.2% of extinction responding respectively). In concordance with behavioral data, mice challenged with saline following cocaine self-administration and extinction exhibited elevated AMPA:NMDA ratios (1.53±0.11) in comparison to control animals (0.98±0.08). However, mice that reinstated drug-seeking behavior following a cocaine (1.00±0.04), stress (0.96±0.08), or cue prime (0.92±0.09) displayed de-potentiated AMPA:NMDA ratios down to control levels. Interestingly, AMPA:NMDA ratios were still potentiated in mice that failed to reinstate drug-seeking behavior (1.41±0.08). Collectively, these data indicate a common neurobiological response - AMPAR depotentiation in the NAc shell - to different stimuli that provoke a reinstatement of drug seeking. Future studies will determine the extent to which this response may modulate or mediate drug seeking.

Disclosures: **S.R. Ebner:** None. **M.C. Hearing:** None. **E.B. Larson:** None. **M.J. Thomas:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.06/L24

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA019666

K02 DA035459

University of Minnesota MnDrive

Breyer-Longden Family Research Fund

Title: Optogenetic self-stimulation of the infralimbic-accumbens pathway: opposing effects of abstinence from repeated cocaine and cocaine re-exposure

Authors: *E. B. LARSON, A. J. ASP, M. ESGUERRA, M. C. HEARING, K. A. SILVIS, M. J. THOMAS;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Mice will actively self-administer optogenetic stimulation of glutamatergic inputs to the nucleus accumbens shell (Stuber et al. 2012; Britt et al. 2012). In drug-naïve animals, optogenetic self-stimulation is largely input-pathway independent, suggesting that glutamate non-discriminately supports behavioral reinforcement (Britt et al. 2012). However, it is unknown how cocaine experience may alter the ability of glutamatergic inputs to the nucleus accumbens shell to promote behavioral reinforcement. We have previously shown that abstinence from repeated cocaine and cocaine re-exposure produce bidirectional synaptic plasticity in the nucleus accumbens shell (Kourrich et al. 2007). Furthermore, while input-specific changes in glutamatergic synaptic function are apparent during cocaine abstinence (Pascoli et al. 2012), the effects of cocaine re-exposure in abstinence on input-specific plasticity is unknown. Therefore, to better understand how input-specific plasticity induced by cocaine experience may alter the ability of excitatory transmission in the NAcSh to reinforce behavior, we compared input-specific optogenetic self-stimulation behavior in animals with different histories of cocaine experience. C57BL/6J mice were infected with channelrhodopsin in either the infralimbic cortex (IL), ventral hippocampus (vHipp), or basolateral amygdala (BLA), and optical fibers were implanted over the NAcSh to allow for selective stimulation of these inputs. Mice were next treated with saline or a cocaine sensitization regimen (15 mg/kg i.p, 5 once daily injections) followed by 10-14 days of abstinence. A spatial optical self-stimulation task was used to assess behavioral reinforcement after cocaine abstinence, or after being re-exposed to cocaine in abstinence. We found that IL-NAcSh self-stimulation was more pronounced in cocaine-abstinent mice compared to drug-naïve controls (10 hz, 5 ms, 5 s max pulse per entry into the “active” zone). Interestingly, cocaine re-exposure dampened IL-NAcSh self-stimulation behavior to levels of control mice. In contrast, self-stimulation of vHipp-NAcSh inputs was enhanced by cocaine abstinence, and further augmented by cocaine re-exposure. Importantly, these different behavioral effects were paralleled by input-specific changes in synaptic plasticity as measured by whole cell patch clamp recordings in medium spiny neurons. Together, our findings suggest that input-specific changes in plasticity with cocaine abstinence and re-exposure directly modify the reinforcing effects of glutamate in the NAcSh.

Disclosures: **E.B. Larson:** None. **A.J. Asp:** None. **M. Esguerra:** None. **M.C. Hearing:** None. **K.A. Silvis:** None. **M.J. Thomas:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.07/L25

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA019666

K02 DA035459

P30 NS062158

Univ. of Minnesota MnDRIVE Fund

Title: Synaptic depotentiation via mGluR5 activation and AMPAR internalization in the nucleus accumbens shell drives cocaine-primed reinstatement

Authors: *M. A. BENNEYWORTH, M. C. HEARING, A. J. ASP, A. E. INGEBRETSON, C. E. SCHMIDT, S. R. EBNER, M. ESGUERRA, M. J. THOMAS;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Relapse after periods of drug abstinence is a major obstacle to long-lasting recovery for many addicts. Understanding the neurobiological processes that incite drug craving and drive relapse has the potential to help target our efforts to treat addiction. The nucleus accumbens (NAc) serves as a critical interface of mesolimbic dopamine circuitry with excitatory cortical afferents in the regulation of motivation and drug seeking. Repeated cocaine exposure potentiates synaptic strength in the NAc medium spiny neurons, which is thought to promote addiction-related behavior. However, the present studies tested the hypothesis that reversal of that augmented synaptic strength, or depotentiation, in the NAc shell is the critical factor in relapse. In support of this hypothesis, we used cocaine conditioned place preference (CPP) behavior and *ex vivo* whole-cell electrophysiology to show that cocaine-primed reinstatement and synaptic depotentiation was disrupted by intra-NAc shell infusion of the tat-GluA23Y "interference" peptide (inhibitor of activity-dependent AMPAR internalization; Ahmadian et al., 2004). Metabotropic glutamate receptor subtype 5 (mGluR5) activation is one mechanism known to promote synaptic depression. Therefore, we investigated whether cocaine-primed reinstatement is driven by an mGluR5-dependent reduction in AMPA-type glutamate receptor signaling. Intra-NAc shell infusion of the mGluR5 antagonist MTEP blocked cocaine-primed reinstatement and corresponding depotentiation, while infusion of the mGluR5 agonist CHPG produced a dose-dependent reinstatement of CPP and depotentiated synaptic strength in the NAc shell. Using an optogenetic approach to look at the role of glutamatergic afferent signaling in the NAc shell, we observed that low frequency blue-light stimulation of the NAc (10 Hz, 5 min, 5ms pulse width) produces reinstatement of CPP in mice infected with AAV-ChR2. Interestingly, this effect was observed when ChR2 was expressed in either the infralimbic cortical or ventral hippocampal projections. This optical stimulation is modeled after electrical stimulation protocols that produce mGluR1/5-dependent LTD (Grueter et al 2010). These findings support a model in which mGluR5-mediated reduction in synaptic GluA2-containing AMPAR function in NAc shell medium spiny neurons can mediate the reinstatement of cocaine-primed behavior.

Disclosures: **M.A. Benneyworth:** None. **M.C. Hearing:** None. **A.J. Asp:** None. **A.E. Ingebretson:** None. **C.E. Schmidt:** None. **S.R. Ebner:** None. **M. Esguerra:** None. **M.J. Thomas:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.08/L26

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA019666

NIH Grant K02 DA035459

The Breyer-Longden Family Research Fund

Title: Bidirectional ethanol-induced synaptic plasticity and reinstatement of place preference following a history of combined ethanol and cocaine exposure

Authors: ***M. ESGUERRA**[1], M. C. HEARING[1], C. E. SCHMIDT[1], A. E. INGEBRETSON[1], T. MACHEDA[1], M. A. BENNEYWORTH[1], M. J. THOMAS[1,2];
[1]Neurosci., Univ. Minnesota, Minneapolis, MN; [2]Psychology, Univ. of Minnesota, Minneapolis, MN

Abstract: Repeated exposure to drugs of abuse induces plasticity at excitatory synapses in the nucleus accumbens (NAc) that are central to the development and persistence of addiction-related behavior. An abundance of data indicates that experience-dependent plasticity in NAc glutamatergic synaptic transmission is primarily expressed via dynamic changes in AMPA-type glutamate receptors (AMPARs), making these receptors a key target for studying how drug experiences modify behavior in models of addiction. In humans, coabuse of drugs is pervasive, with concurrent cocaine and alcohol use being a frequently abused combination; however, to date, knowledge of drug-induced synaptic plasticity is often limited to single-drug regimens. Using a model of conditioned-place preference (CPP), our studies test the hypothesis that co-administration of cocaine and ethanol modulates NAc synaptic plasticity at glutamatergic afferents to MSNs in a way that may explain the potent effects of this drug combination on behavior. In wild-type mice, co-administration of cocaine (7.5 mg/kg ip) and ethanol (2 g/kg ip) as the conditioned stimulus reliably produced a modest conditioned place preference which extinguished rapidly. On re-exposure to ethanol alone (2 g/kg) after extinction, animals showed divergent behavioral responses, with one subpopulation displaying robust reinstatement of preference for the conditioned stimulus (580.40 ± 66.8 sec CS bias, n=16), while another showed dramatic aversion to the conditioned stimulus (390.97 ± 90.4 sec anti-CS bias, n=10) after ethanol primed reinstatement. A subset of mice was sacrificed immediately after the ethanol

reinstatement test to examine alterations in synaptic AMPA receptor-mediated transmission in the NAc using whole-cell recordings from MSNs in acute slices. In the NAc shell in animals with a strong preference for the conditioned stimulus, the amplitudes of miniature excitatory postsynaptic currents (mEPSCs) were similar to saline controls. However in animals with inverted preference, mEPSC amplitudes were increased by >16%, suggesting that synaptic plasticity in NAc may underlie aversion to the CS in this population. Our results suggest that re-exposure to ethanol induces bidirectional synaptic plasticity in nucleus accumbens, in parallel with divergent behavioral responses. Ongoing experiments will examine how cocaine modulates this divergence, and whether motor and sensory impairment contribute to bimodal reinstatement after ethanol priming.

Disclosures: **M. Esguerra:** None. **M.C. Hearing:** None. **C.E. Schmidt:** None. **A.E. Ingebretson:** None. **T. Macheda:** None. **M.A. Benneyworth:** None. **M.J. Thomas:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.09/L27

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA019666

K02 DA035459

Breyer-Longden Family Research Fund

Title: Cellular mechanisms and timing of cocaine-induced synaptic depotentiation in the nucleus accumbens

Authors: ***A. E. INGEBRETSON**[1], M. C. HEARING[2], S. R. EBNER[2], M. J. THOMAS[2]; [1]Neurosci., [2]Univ. of Minnesota, Minneapolis, MN

Abstract: Repeated exposure to cocaine can promote drug-seeking and -taking behavior. In rodent addiction models, enduring changes in glutamatergic synaptic transmission in the nucleus accumbens (NAc) appear to be important in driving this behavior. Repeated exposure to cocaine is known to potentiate AMPAR-mediated signaling in medium-spiny neurons (MSNs) in the NAc shell 10-14 days following the last drug exposure. This increase in synaptic function is reversed (or, “depotentiated”) by a single re-exposure to cocaine. However, the cellular mechanisms and the detailed timing of this drug-evoked plasticity are not well characterized. We investigated the onset and duration of NAc synaptic depotentiation as well as potential underlying mechanisms driving this plasticity, focusing on region-specific changes (shell versus core). Extending previous findings (which focused on NAc shell), we observed that in the NAc

core, AMPAR-mediated transmission was augmented following 10-14 d withdrawal, and reversed 24 h following drug re-exposure. In both NAc shell and core MSNs, challenge-induced depotentiation of AMPAR-transmission returned to control levels 5 d following drug re-exposure, indicating that this plasticity is transient. To determine the underlying mechanisms of the drug-induced depotentiation, we employed a novel *ex vivo* "bath challenge" model of drug re-exposure. *Ex vivo* re-exposure to cocaine induced depotentiation of AMPAR-mediated synaptic transmission in NAc shell and core MSNs within 30 min of drug exposure. In the NAc, activation of postsynaptic group I metabotropic glutamate receptors (mGluRs) has been shown to promote reduced presynaptic glutamate release probability and increased trafficking of AMPA-type glutamate receptors (McCutcheon et al., 2011; Robbe et al., 2002). Consistent with this, in the NAc shell, prior exposure to the mGluR5 antagonist MTEP blocked the *ex vivo* cocaine-induced reductions in mEPSC amplitude but not frequency_presumed measures of posysnaptic and presynaptic function respectively. In contrast, in the NAc core, prior exposure to MTEP did not influence the *ex vivo* cocaine-induced reductions on mEPSC amplitude or frequency. Interestingly, blockade of endocannabinoid signaling using the eCB1 receptor antagonist (SR-141716A), blocked cocaine-induced reductions in mEPSC frequency but not amplitude, suggesting the existence of multiple, independent forms of synaptic plasticity in response to drug re-exposure during abstinence. Using this approach to further delineate mechanisms and timing of drug-induced plasticity will help elucidate the neurobiology of susceptibility to addiction relapse.

Disclosures: A.E. Ingebretson: None. **M.C. Hearing:** None. **S.R. Ebner:** None. **M.J. Thomas:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.10/L28

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA23206

Title: NMDA receptor subtypes control maturation of cocaine-generated silent synapse in nucleus accumbens

Authors: *Y. WANG[1], M. OTAKA[2], P. MU[2], M. ISHIKAWA[2], J. WANG[2], O. M. SCHLÜTER[4], Y. DONG[2], Y. H. HUANG[3];
[2]Neurosci., [3]Psychiatry, [1]Univ. of Pittsburgh, Pittsburgh, PA; [4]4Molecular Neurobio. and Cluster of Excellence "Nanoscale Microscopy and Mol., European Neurosci. Inst., Göttingen, Germany

Abstract: Exposure to cocaine induces adaptive molecular and cellular changes in the forebrain nucleus accumbens (NAc), leading to subsequent drug craving, drug seeking and drug taking behaviors. Using repeated i.p. injection procedure, we previously demonstrate in the NAc that exposure to cocaine generates AMPA receptor-silent excitatory synapses, which are enriched in GluN2B-containing NMDA receptors. These cocaine-generated silent synapses are likely immature synaptic contacts that evolve into fully functional synapses after cocaine withdrawal. Similar to the non-contingent procedure, after cocaine self-administration (2 h/session/day x 5 d) we also observed a substantial increase in the level of silent synapses in the NAc, enriched in GluN2B-containing NMDARs. Furthermore, the increased level of GluN2B-containing NMDARs returned to the basal levels after 7-day withdrawal, accompanied by the maturation of cocaine-generated silent synapses. In a mouse line in which the CaMKII-binding to GluN2B was compromised, exposure to cocaine generated silent synapse in the NAc, but these silent synapses remain silent after cocaine withdrawal without undergoing the maturation process. These results lead to our hypothesis that GluN2B-containing NMDARs control the maturation of these cocaine-generated silent synapses. Our future experiments will test this hypothesis by determining how GluN2B and its coupled signaling regulate maturation of cocaine-generated silent synapses and whether targeting NAc GluN2B is a strategy to reverse cocaine-induced circuitry remodeling.

Disclosures: **Y. Wang:** None. **M. Otaka:** None. **P. Mu:** None. **M. Ishikawa:** None. **J. Wang:** None. **O.M. Schlüter:** None. **Y. Dong:** None. **Y.H. Huang:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.11/L29

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH MH101147

DA035805

DA029565

DA023206

DA034856

DA030379

Title: Cocaine self-administration generates silent synapses in thalamus to nucleus accumbens projection

Authors: *P. A. NEUMANN[1,3], N. GRAZIANE[2], Y. H. HUANG[2], W. XU[3], S. R. SESACK[1], E. J. NESTLER[4], O. M. SCHLÜTER[5], Y. DONG[1];
[2]Psychiatry, [1]Univ. of Pittsburgh, Pittsburgh, PA; [3]Stanford Univ., Palo Alto, CA; [4]Fishberg Dept. of Neurosci. and Friedman Brain Inst., Mount Sinai Sch. of Med., New York, NY; [5]Mol. Neurobio., European Neurosci. Inst., Göttingen, Germany

Abstract: Drug Addiction is characterized by maladaptive changes in signaling between brain regions which regulate rewards and motivated behaviors. The paraventricular thalamic nucleus (PVT) is a brain region which sends direct projections to the nucleus accumbens (NAc) and is increasingly being linked to addiction-related behaviors. We sought to characterize the molecular and cellular changes within the PVT-to-NAc pathway in response to cocaine self-administration. We used virally-mediated channelrhodopsin expression in the PVT of rats to isolate fibers from the PVT and recorded from neurons in the NAc shell. We found that cocaine self-administration increases silent synapses within the PVT-to-NAc pathway. Additionally, calcium-permeable AMPARs are present at PVT-to-NAc synapses under basal conditions, but are not additionally recruited to maturing silent synapses. Cocaine self-administration also leads to a greater probability of presynaptic vesicle release, which persists though long-term withdrawal. Disrupting PVT neurons that project to the NAc works to inhibit the acquisition of cocaine self-administration. These results characterize an array of cellular and molecular signaling changes along the PVT-to-NAc pathway in the context of cocaine exposure and demonstrate that this pathway plays a significant role in the acquisition of cocaine self-administration.

Disclosures: P.A. Neumann: None. **N. Graziane:** None. **Y.H. Huang:** None. **W. Xu:** None. **S.R. Sesack:** None. **E.J. Nestler:** None. **O.M. Schlüter:** None. **Y. Dong:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.12/L30

Topic: C.17. Drugs of Abuse and Addiction

Support: R00-DA031699

Title: Cocaine exposure alters thalamo-accumbens synapses

Authors: *M. E. JOFFE[1], B. A. GRUETER[2];
[1]Pharmacol., [2]Anesthesiol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Dysregulation of the mesolimbic system is a hallmark of the pathophysiology of drug addiction and other reward-related psychiatric diseases. The nucleus accumbens (NAc) is well known as a key biological substrate that modulates the incentive and hedonic properties of drugs of abuse. At least 90% of the neurons in the NAc are GABAergic medium spiny neurons

(MSNs). The MSNs, which provide the output of the NAc, can be divided into two classes anatomically and biochemically: one class of MSNs project primarily to the midbrain DA areas and express D1 DA receptors, while the other MSNs project to the ventral pallidum and express D2 DA and A2A adenosine receptors. MSNs rest at relatively hyperpolarized membrane potentials; therefore excitatory drive is essential to governing the output of the NAc and subsequent complex behavioral outcomes. The prefrontal cortex, ventral hippocampus, and basolateral amygdala, provide major excitatory inputs to the NAc and each have demonstrated relevance to drug exposure and subsequent behavioral abnormalities. However, the midline thalamic nuclei (thal) also send dense glutamatergic projections to the NAc core, but little is known about how these synapses may subserve conditioned reward-related behaviors. To gain a better understanding of the physiology and plasticity at thal-NAc synapses we virally expressed channel rhodopsin in the midline thalamic nuclei of D1-tdtomato BAC transgenic mice and performed targeted whole-cell patch-clamp electrophysiology in the NAc core. These experiments revealed that under basal conditions, thal-D1(+) and thal-D1(-) exhibit differential synaptic properties as assessed by ratiometric measures of ionotropic glutamate receptor (NMDAR and AMPAR) function. We then proceeded to assess the consequences of *in vivo* cocaine exposure on the thal-NAc circuit, following 2 weeks of abstinence from cocaine exposure. These experiments uncovered adaptations at both thal-D1(+) and thal-D1(-) synapses that are likely important for the behavioral changes observed at this stage of cocaine exposure. These novel findings point towards new potential avenues for DBS- or pharmacotherapy-assisted drug addiction treatments.

Disclosures: **M.E. Joffe:** None. **B.A. Grueter:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.13/L31

Topic: C.17. Drugs of Abuse and Addiction

Support: CRCNS program, R01DA-038890

MURI N00014-10-1-0198

Title: Corticostriatal LTP is modulated by direct pathway co-release of dynorphin

Authors: ***S. L. HAWES**, K. T. BLACKWELL;
George Mason Univ., Fairfax, VA

Abstract: Synaptic plasticity adjusts behavior adaptively in the case of skill learning, or maladaptively in the case of addiction. Just as dopamine plays a critical role in synaptic plasticity underlying normal skill learning and addiction, endogenous and exogenous opiates modulate

learning and addiction related striatal plasticity. While the effect of opioids on LTD has been characterized, their effect on LTP remains unknown. This study investigates the effect of opioid neuropeptides co-released by direct pathway (D1) MSNs on corticostriatal LTP. We cross Ai32 female mice, which contain genes for channel rhodopsin 2 and EYFP downstream of a loxP-flanked STOP cassette, to Tg(Drd1a-cre) EY217 male mice. This generates Cre-positive offspring with optically-excitable D1-MSNs. In striatal brain slice from these offspring, we supply blue light through a 40x submersion objective to drive endogenous co-release from the D1-MSN population coincident with theta-burst stimulation of cortical afferents. First, we demonstrate that this afferent theta-burst stimulation (10.5Hz with 50Hz intra-burst) evokes corticostriatal LTP using the whole cell patch recording technique. Subsequently we show that optical activation of D1-MSNs during induction impairs LTP. We hypothesize that the opioid neuropeptide dynorphin, co-released exclusively by D1-MSNs, is responsible for this reduced TBS LTP. Dynorphin-activated kappa opioid receptors reside presynaptically on dopaminergic afferents and are capable of negatively regulating dopamine release, previously demonstrated to be essential for LTP in dorsomedial striatum. In support of this hypothesis, we demonstrate full rescue of LTP induced with optical activation of D1-MSNs by bath application of the kappa opioid receptor antagonist nor-Binaltorphimine dihydrochloride (1μM). Our findings illustrate a physiological phenomenon whereby heightened D1-MSN activity can regulate corticostriatal plasticity. Ongoing experiments will establish whether this indeed functions through regulation of dopamine availability, and will address whether LTP modulation by D1-MSN co-release differs between direct and indirect pathway MSNs. Our findings have important implications for learning in addictive states marked by elevated direct pathway activation.

Disclosures: **S.L. Hawes:** None. **K.T. Blackwell:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.14/L32

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Intramural Research Program

Title: Incubation of methamphetamine craving is associated with selective increases in expression of BDNF and TrkB, glutamate receptors, and epigenetic enzymes in cue-activated Fos-expressing dorsal striatal neurons

Authors: ***X. LI**[1], F. RUBIO[1], T. ZERIC[1], J. M. BOSSERT[1], S. KAMBHAMPATI[1], H. M. CATES[2], P. J. KENNEDY[3], Q.-R. LIU[1], R. CIMBRO[4], B. T. HOPE[1], E. J. NESTLER[2], Y. SHAHAM[1];
[1]Behavioral Neurosci. Res. Br., Natl. Inst. On Drug Abuse, Baltimore, MD; [2]Fishberg

department of Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Dept. of Psychology, Univ. of California Los Angeles, Los Angeles, CA; [4]John Hopkins Univ., Division of Rheumatology, MD

Abstract: Cue-induced methamphetamine seeking progressively increases after withdrawal (incubation of methamphetamine craving) but the underlying mechanisms are largely unknown. We determined whether this incubation is associated with alterations in candidate genes in dorsal striatum (DS), a brain area implicated in cue- and context-induced drug relapse. We first measured mRNA expression of 24 candidate genes in whole DS extracts after short (2 d) or prolonged (1 month) withdrawal in rats following extended access methamphetamine or saline (control condition) self-administration (9-h/day; 10-days). We found minimal changes. Next, using FACS, we compared gene expression in Fos-positive dorsal striatal neurons, which were activated during 'incubated' cue-induced drug-seeking tests after prolonged withdrawal, with non-activated Fos-negative neurons. We found significant increases in mRNA expression of immediate early genes (IEGs: Arc, Egr1), Bdnf and its receptor (Trkb), glutamate receptor subunits (Gria1, Gria3, Grm1), and epigenetic enzymes (Hdac3, Hdac4, Hdac5, GLP, Dnmt3a, Kdm1a) in the Fos-positive neurons only. Using RNAscope® to determine striatal sub-region and cell-type specificity of the activated neurons, we measured co-labeling of Fos with Drd1 and Drd2 in three DS sub-regions. Fos expression was neither sub-region nor cell-type specific (52.5% and 39.2% of Fos expression co-labeled with Drd1 and Drd2, respectively). Finally, we found that DS injections of SCH23390, a D1-family receptor antagonist known to block cue-induced Fos induction, decreased 'incubated' cue-induced methamphetamine seeking after prolonged withdrawal. Results demonstrate a critical role of DS in incubation of methamphetamine craving and that this incubation is associated with selective gene-expression alterations in cue-activated D1- and D2-expressing DS neurons.

Disclosures: **X. Li:** None. **F. Rubio:** None. **T. Zeric:** None. **J.M. Bossert:** None. **S. Kambhampati:** None. **H.M. Cates:** None. **P.J. Kennedy:** None. **Q. Liu:** None. **R. Cimbro:** None. **B.T. Hope:** None. **E.J. Nestler:** None. **Y. Shaham:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.15/L33

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA AA020501

Title: Distinct glutamatergic inputs to nucleus accumbens medium spiny neurons control synaptic plasticity and its modulation by alcohol

Authors: *X. JI, S. SAHA, G. E. MARTIN;
Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: In the core nucleus accumbens (NAc), a brain region involved in drug reward, it is accepted that long-lasting changes of synaptic strength mediate acute and chronic effects of most drugs of abuse, including alcohol. Yet, very little is known about this cellular phenomenon. Of particular interest is the fact that core NAc medium spiny neurons (MSNs) receive glutamatergic inputs from distinct brain regions, i.e. the prefrontal cortex, the amygdala and the hippocampus, each informing the NAc with a different types of information (e.g. spatial, emotional and cognitive). However, their respective role in shaping synaptic plasticity remains unknown. The role of each pathway in mediating the effects of alcohol on synaptic plasticity also has never been examined. Using patch clamp recordings and the optogenetic techniques to independently recruit each input, we show that stimulation of prefrontal cortex afferents consistently evoked spike-timing dependent tLTD but failed to induce tLTP. In contrast, stimulation of amygdala and hippocampal inputs led to both tLTP and tLTD. Comparison of the basic properties of AMPA/NMDA-mediated synaptic transmission revealed markedly different NMDA receptor properties and probability of release at amygdala, cortical and hippocampal synapses. Importantly, low 20 mM acute ethanol powerfully inhibited tLTP but had only a mild effect on tLTD. This study suggests that glutamatergic inputs to NAc medium spiny neurons are not identical, determine synaptic plasticity and its regulation by alcohol.

Disclosures: X. Ji: None. **S. Saha:** None. **G.E. Martin:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.16/L34

Topic: C.17. Drugs of Abuse and Addiction

Support: China National Science Foundation (Project No. 31170990)

China National Science Foundation (Project No. 81100992）

Title: Cognitive behavioral therapy rectified the altered functional connectivity of ventral striatum in Internet gaming disorder group

Authors: *S.-S. MA[1], J.-T. ZHANG[1], C.-S. R. LI[2], L. LIU[3], L.-J. WANG[1], B. LIU[1], Y.-W. YAO[1], X.-Y. FANG[3];
[1]Natl.Key Lab.of Cognitive Neurosci.& Learning& IDG/McGovern Inst. for Brain Res., Beijing Normal Univ., Beijing, China; [2]Dept. of Psychiatry, Yale University,School of Med., New Haven, CT; [3]Inst. of Developmental Psychology.,Beijing Normal Univ., Beijing, China

Abstract: Internet gaming disorder (IGD) is associated with the altered cortico-striatal activities. Resting-state functional connectivity (rsFC) of functional magnetic resonance imaging (fMRI) signals is a non-invasive and systems-level approach to assess functional interaction between brain areas and provides a powerful tool to identify biological markers of various psychiatric disorders, including addiction. Cognitive Behavioral Therapy (CBT) has been consistently found to be effective in ameliorating adverse impacts of Internet addiction. However, little is known about the neural bases of CBT and whether it can remediate cortico-striatal connectivity. We firstly conducted a cross-section study to examine the altered rsFC of the ventral striatum in 74 adolescent IGDs compared with 41 age- and gender-matched healthy controls (HCs). Then in the follow-up intervention study, 39 of the 74 IGDs were scanned twice, including 23 IGDs who accepted CBT (IGD-intervention) and 16 IGDs without CBT (IGD-control). IGDs showed greater functional connectivity of ventral striatum (NAc) with left inferior parietal gyrus, right inferior frontal gyrus and left middle frontal gyrus, which were positively associated with the clinical assessments. All of these indices showed more decrease in IGD-intervention group than in IGD-control group. Moreover, there is a significant interaction between intervention and group in functional connectivity between ventral striatum (NAc) and left inferior parietal gyrus, along with the clinical assessments. Results suggested that CBT can moderate the abnormality of cortico-ventral striatal connectivity, and the functional connectivity between ventral striatum (NAc) and left inferior parietal gyrus may be a stable and sensitive biomarker for diagnosis of IGD and detection of the effects of CBT.

Disclosures: S. Ma: None. **J. Zhang:** None. **C.R. Li:** None. **L. Liu:** None. **L. Wang:** None. **B. Liu:** None. **Y. Yao:** None. **X. Fang:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.17/L35

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA031747

Title: Neuroplasticity associated with individual sensitivity to a natural reward following cocaine exposure

Authors: *B. O'DONOVAN, P. I. ORTINSKI;
Pharmacology, Physiol. and Neurosci., Univ. of South Carolina, Columbia, SC

Abstract: Withdrawal from psychostimulants such as cocaine and amphetamine has been associated with reduced motivation for natural rewards in both rats and humans. Rats exhibit individual differences in their consumption of naturally rewarding sucrose. There is evidence to

suggest that rats with a high preference for sweet substances differ from rats with a low preference in their response to psychostimulants. However, a potential difference between these groups in neuronal plasticity in the NAc in response to cocaine has yet to be fully explored. This study examined the effect of a 'binge' cocaine treatment on the motivation to achieve sucrose reward and associated changes in neuronal excitability in the NAc shell of these animals. Rats were trained to respond for a sucrose reward on a progressive ratio (PR) schedule of reinforcement and divided into high and low responders based on their breakpoints. Breakpoints were then measured over 5 days of saline or 'binge' cocaine treatments (3 daily injections at 1 hour intervals, 15mg/kg i.p.) and for 2 days after the termination of drug delivery. Following the final PR session, slices containing NAc were prepared and whole-cell patch clamp recordings were taken in the NAc shell. In cocaine treated rats, breakpoints were reduced during the 5 days of cocaine treatment and up to 2 days following the termination of treatment. No difference in the magnitude of the reduction was seen between the high and low responder groups. In saline treated rats breakpoints remained stable for the duration of the experiment. Whole-cell patch clamp recordings from medium spiny neurons in the NAc shell indicated a pattern of differences between the high and low responder groups on measures of action potential firing and excitatory synaptic strength. These results demonstrate that exposure to the same cocaine regime reveals broad variability in excitability of NAc neurons associated with individual motivation for a natural reward. We are now exploring whether individual sucrose preference in the absence of cocaine similarly co-varies with measures of membrane and synaptic excitability.

Disclosures: B. O'Donovan: None. **P.I. Ortinski:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.18/L36

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/1K99-DA038706-01

R01 DA019666

Title: Reversal of morphine-induced cell-type specific synaptic plasticity in the nucleus accumbens shell blocks reinstatement

Authors: *M. C. HEARING, A. INGEBRETSON, S. EBNER, C. SCHMIDT, R. FISCHER, A. ASP, M. THOMAS;
Dept. of Neurosciences, Univ. of Minnesota, Minneapolis, MN

Abstract: Repeated exposure to drugs of abuse such as cocaine, induces plasticity at excitatory synapses in the nucleus accumbens (NAc). However, little is known of opiate-induced synaptic

plasticity in NAc and its relevance to drug-seeking. Medium spiny neurons (MSNs), the principal NAc cell-type, typically express either the dopamine receptor 1 (D1-MSNs) or dopamine receptor 2 (D2-MSNs), the presence of which plays a role in determining cell physiology and contribution to drug-related behaviors. To identify morphine-induced plasticity in glutamatergic signaling in the NAc, we used BAC transgenic mice expressing fluorescent proteins under the control of the D1R or D2R promoter, and a model of morphine sensitization to identify adaptations in glutamatergic signaling within the NAc shell (and core) following 10-14 d withdrawal. In the NAc shell, AMPAR/NMDAR (A/N) ratios and the amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) are increased in D1R- but not D2R-MSNs, indicating synaptic potentiation in the D1R-MSNs. To test for morphine-induced changes in the subunit composition of synaptic AMPARs in D1R-MSNs, we assessed current-voltage relationships of evoked EPSCs and the effect of bath application of 1-naphthylacetylsperimine (Naspm), a selective blocker of GluA2-lacking AMPA receptors. The rectification index increased and Naspm decreased evoked EPSC amplitude in neurons recorded from the morphine-exposed mice_indicators of the presence of GluA2-lacking AMPARs. Given the morphine-induced changes in mEPSC frequency, we used paired-pulse stimulation to test for changes in glutamate release probability, we find significant increases and decreases in release probability within D1R-MSNs and D2-MSNs respectively. We next explored whether reversal of this plasticity is able to prevent drug-associated behavior using a conditioned place preference model. We found that repeated administration of the antibiotic, Ceftriaxone, which up-regulates expression of the glutamate transporter GLT-1, during withdrawal reversed increases in D1-MSNs AMPAR signaling, enhanced signaling in D2-MSNs, and blocked reinstatement of morphine place preference. Furthermore, using an AAV viral vector expressing hM3Dq (Gq) DREADD under control of the glial fibrillary acidic protein (GFAP), we demonstrated that repeated activation of DREADD-mediated signaling in the NAc shell during withdrawal attenuates reinstatement of morphine place preference. These data suggest that cell-type specific adaptations in NAc shell MSN synaptic strength are critical pathophysiological mechanisms underlying morphine-associated behavior.

Disclosures: **M.C. Hearing:** None. **A. Ingebretson:** None. **S. Ebner:** None. **C. Schmidt:** None. **R. Fischer:** None. **A. Asp:** None. **M. Thomas:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.19/L37

Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA037257 (D.M.D)

NIAAA T-32-AA007583

GM09545902

Title: Drebrin signaling mediates opiate-induced plasticity in the nucleus accumbens

Authors: *J. A. MARTIN[1], Z.-J. WANG[1], M. HUMBY[1], A. CACCAMISE[1], L. E. MUELLER[1], R. NEVE[2], A. M. GANCARZ[1], D. M. DIETZ[1];
[1]State Univ. of New York At Buffalo, Buffalo, NY; [2]MIT, Cambridge, MA

Abstract: Opiate addiction has dramatically increased, becoming a worldwide epidemic with great societal and financial burdens. Drug addiction, defined as a chronic relapsing disease, involves the 'rewiring' of the brain through long-term changes, such as structural plasticity, in several key regions of the mesolimbic dopaminergic pathway. There is a great deal of evidence demonstrating that chronic psychostimulant exposure increases the number of dendritic spines on medium spiny neurons in the nucleus accumbens (NAc). In contrast, exposure to opiates, such as morphine and heroin which similarly induce behavioral sensitization, leads to a decrease in dendritic spine density. However, there are minimal data regarding the cellular neurobiology that regulates this opiate-induced plasticity. Following both morphine sensitization and heroin self-administration there is a decreased expression of the actin binding protein drebrin in the NAc. This decrease in drebrin results from an increase in HDAC2 expression and binding at the promoter of the transcriptional start site, accompanied by a decrease in pan-H3 acetylation. Overexpression of drebrin blunted the development of morphine sensitization (5 mg/kg, i.p.) and attenuated the expression of sensitization following a morphine challenge (2.5 mg/kg, i.p.) compared to HSV-GFP controls. In order to determine the role of drebrin in drug relapse, animals were trained to self-administer heroin (0.02 mg/kg/inf). Interestingly, following heroin self-administration, overexpression of drebrin in the NAc significantly decreased responding during heroin-primed reinstatement (0.25 mg/kg, s.c.), but not cue-induced reinstatement. Finally, overexpression of HDAC2 leads to a potentiation of behavioral response to low doses of morphine. Taken together, these data suggest that epigenetic regulation of drebrin is functionally regulated following exposure to opiates and may be a key molecular mechanism underlying opiate-induced behavioral plasticity.

Disclosures: J.A. Martin: None. **Z. Wang:** None. **M. Humby:** None. **A. Caccamise:** None. **L.E. Mueller:** None. **R. Neve:** None. **A.M. Gancarz:** None. **D.M. Dietz:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.20/L38

Topic: C.17. Drugs of Abuse and Addiction

Support: VA Merit Review BX09-008

Title: Dopamine D1 agonist treatment alters opiate reward extinction and accumbal dendritic complexity

Authors: *G. B. KAPLAN[1], K. L. KOBRIN[2], D. ARENA[3], S. C. HEINRICHS[3];
[1]Psychiatry and Pharmacol., [2]Pharmacol., Boston Univ. Sch. Med/VA Boston Healthcare, Boston, MA; [3]Res., VA Boston Healthcare Syst., Boston, MA

Abstract: The dopamine D1 receptor (D1R) mediates drug reward and morphology of accumbal neurons. We used a morphine conditioned place preference (CPP) mouse model to study the role of D1R in context-reward associative learning and related accumbal dendritic morphology changes. To acquire CPP, a morphine group received saline or morphine 10 mg/kg s.c. on alternate days, while CPP controls received saline on all days, immediately before 8 daily place conditioning sessions. Post-conditioning, morphine CPP mice expressed significantly higher place preference than saline mice. We examined the effects of D1R agonist on morphine reward extinction. To induce extinction, mice were repeatedly exposed to the place conditioning environment in a drug-free state. Morphine-conditioned mice were divided into three groups, receiving s.c.saline, 0.5 mg/kg SKF81297 (D1R agonist), or 0.8 mg/kg SKF81297 immediately after each extinction session, while saline sham-conditioned controls received saline. After every two days of extinction training mice were tested for place preference. D1R agonist treatment inhibited extinction of place preference in a dose-dependent manner. After the third post-extinction preference test, the 0.8 mg/kg SKF81297 group's mean preference score was twice as high as the mean preference score of the group of morphine-conditioned mice receiving saline during extinction. One day after the final place preference test brains were isolated and processed using Golgi-Cox staining followed by digital tracing of accumbal neuron morphology. Neurons of the accumbens core from mice treated with 0.8 mg/kg SKF81297 that were prevented from undergoing CPP extinction-related changes had signficantly greater dendritic complexity and spine density than controls that achieved CPP extinction. No differences were found in the accumbens shell. D1R agonist appears to activate drug reward memories, reverse extinction, and increase accumbal core dendritic complexity.

Disclosures: G.B. Kaplan: None. **K.L. Kobrin:** None. **D. Arena:** None. **S.C. Heinrichs:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.21/L39

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of chronic forced exercise on the mesolimbic dopamine pathway: Implications for addiction

Authors: *L. S. ROBISON[1], A. TUCCI[1], J. STAMOS[3], M. ANANTH[2], P. K. THANOS[1]; [1]Psychology, [2]Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY; [3]Psychology, Rutgers Univ., New Brunswick, NJ

Abstract: Substance abuse disorders affect over 20 million people in the United States and cost the nation over $700 billion annually. Therefore, identifying means of reducing the propensity for substance abuse is of great interest. Exercise has been identified as a natural and cost-effective means of both preventing and treating substance abuse. Both clinical and preclinical studies have found that exercise is associated with reduced behavioral reactivity, initiation of drug use, progression of use to addiction, withdrawal symptoms, and likelihood of relapse. Less is known, however, regarding the underlying neurobiological mechanisms driving these changes in behavior. Research has shown that vulnerability for substance abuse may be attributable to "reward deficiency", mediated by altered functioning of the brain's mesolimbic dopamine pathway, which functions in reinforcing both natural and drug rewards. One possibility is that exercise may alter the mesolimbic dopamine pathway in such a way to make drugs of abuse less salient and/or rewarding. Chronic use of drugs and vulnerability to substance abuse has been associated with an increase in excitatory dopamine D1 receptors (D1R) and a decrease in inhibitory dopamine D2 receptors (D2R). Therefore, it was hypothesized that exercise may decrease D1R and/or increase D2R in reward-related regions. Male and female Lewis rats were split into exercise and sedentary groups at 8 weeks of age. Exercise rats were run on a treadmill at 10m/min, five days per week, for six weeks. Sedentary rats received no exercise beyond normal cage ambulation. Following treatment, rats were euthanized and brains were flash-frozen until being cryosectioned and bound with [3H]SCH 23,390, [3H]spiperone, and [3H]WIN55,428 to quantify levels of D1R, D2R, and the dopamine transporter (DAT), respectively. ImageJ software was used to outline and quantify receptor and transporter levels in regions of interest, including the caudate putamen (split into four quadrants: dorsomedial, dorsolateral, ventromedial, and ventrolateral), nucleus accumbens core and shell, olfactory tubercle, and substantia nigra. Results show that exercise attenuates D1R binding in the olfactory tubercle and shell of the nucleus accumbens, while this trend approaches significance in the substantia nigra. Preliminary data suggests that exercise may also increase D2R binding across several regions measured. Analysis of D2R and DAT binding continues. These findings thus far support the hypothesis that exercise results in changes in the mesolimbic pathway that could mediate exercise-induced changes in drug-seeking behavior.

Disclosures: L.S. Robison: None. **A. Tucci:** None. **J. Stamos:** None. **M. Ananth:** None. **P.K. Thanos:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.22/L40

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01DA10355

NIH Grant R01MH61460

Title: Dopamine-acetylcholine interplay in the basal ganglia modulates AMPA glutamate receptors and behavior

Authors: *B. XUE, D.-Z. JIN, L.-M. MAO, J. WANG;
Dept. of Basic Med. Sci., UMKC Sch. of Med., Kansas City, MO

Abstract: Dopamine (DA) and acetylcholine (ACh) converge onto the protein kinase A (PKA) pathway in medium spiny neurons of the striatum and pyramidal output neurons of the medial prefrontal cortex (mPFC) to control the excitability of these neurons and thus behavior, although underlying molecular mechanisms are less clear. Here we measured phosphorylation of AMPA receptors (AMPARs) at a PKA site (S845) as an activation indicator of AMPARs in adult rat brains *in vivo* to explore how DA and ACh interact to modulate AMPARs and behavior. In a series of pharmacological experiments, we characterized GluA1 S845 responses to DA D1 receptor (D1R), D2 receptor (D2R) or muscarinic M4 receptor (M4R) agents in DA responsive regions (striatum and mPFC). The S845 responses support a local multitransmitter interaction model in which D2Rs inhibited an intrinsic inhibitory element mediated by M4Rs to enhance the D1R efficacy in modulating AMPARs. Consistent with this, selective activation of M4Rs through a positive allosteric modulator (PAM) resumed the cholinergic inhibition of D1Rs. In addition, D1R and D2R coactivation recruited GluA1 and PKA preferentially to extrasynaptic sites. Behaviorally, the M4R PAM inhibited the motor responsivity to co-injected D1R/D2R agonists. Our *in vivo* data support the existence of differential DA-ACh balances in the striatum and mPFC which actively modulate GluA1 AMPARs and behavioral sensitivity to changing DA input. A central signaling pathway in these balances involves PKA-dependent and S845-regulated trafficking of GluA1.

Disclosures: **B. Xue:** None. **D. Jin:** None. **L. Mao:** None. **J. Wang:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.23/L41

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH R01AA016022

NIH/NIAAA DICBR

Title: Dopamine release and cocaine sensitivity differ between striosome and matrix compartments of the striatum

Authors: *A. G. SALINAS[1,2], M. I. DAVIS[1], D. M. LOVINGER[1], Y. MATEO[1];
[1]Natl. Inst. On Alcohol Abuse and Alcoholism, Rockville, MD; [2]George Mason Univ., Fairfax, VA

Abstract: The striatum is involved in a number of neural processes including learning and action control. Two major output pathways from striatum have been characterized: the direct and indirect pathways, which consist of D1 and D2 dopamine receptor-enriched neurons, respectively. The striatum can also be classified into striosome and matrix compartments, based on the expression of several proteins, including the mu opioid receptor, dopamine transporter (DAT), and Nr4a1 (nuclear receptor subfamily 4, group A, member 1). A number of functional differences between the striosome and matrix compartments have been implicated in neurological disorders including Parkinson's disease and addiction. Given the importance of dopamine signaling and the differences between striosome and matrix compartments in models of these conditions, we hypothesized that dopamine release would differ between striosome and matrix compartments. To test this hypothesis, we used Nr4a1-GFP mice to identify striosomes and fast scan cyclic voltammetry to measure evoked dopamine overflow from adjacent striosome and matrix compartment pairs. We found that electrically evoked dopamine overflow in striosomes in the dorsal striatum was reduced approximately 35% compared to the matrix compartment. We further found that in the ventral striatum, this pattern was reversed, such that evoked dopamine overflow in striosomes was approximately 64% greater than in the matrix compartment. We then examined three common mechanisms involved in modulating dopamine overflow. We started by examining the effect of quinpirole, a D2 dopamine receptor agonist, on autoreceptor-mediated inhibition of dopamine overflow and found no differences between compartments, neither in dorsal nor in ventral striatum. To examine the contribution of nicotinic receptors, we treated slices with DHBE and found that antagonism of nicotinic acetylcholine receptors inhibited dopamine overflow to a similar degree in both compartments. We then examined the role of DAT in the differences in dopamine release between compartments and found that cocaine enhanced dopamine overflow in striosomes to a greater degree than in the matrix at several concentrations. Modeling of the dopamine overflow kinetics showed that cocaine inhibited dopamine uptake in the matrix compartment to a greater degree than in striosomes. This difference in cocaine sensitivity was limited to the dorsal striatum. Together these findings demonstrate a strict regulation of striosomal dopamine relative to the matrix. The significance of this regulation remains uncertain but has implications for dopamine-related neurological disorders and addiction.

Disclosures: **A.G. Salinas:** None. **M.I. Davis:** None. **D.M. Lovinger:** None. **Y. Mateo:** None.

Poster

320. Striatal Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 320.24/L42

Topic: C.17. Drugs of Abuse and Addiction

Title: Sleep disturbances predict reductions in striatal D2R availability in cocaine abusers

Authors: *C. E. WIERS[1], E. SHUMAY[2], E. CABRERA[3], E. SKARDA[2], E. SHOKRI-KOJORI[2], S. CUNNINGHAM[2], C. WONG[2], D. TOMASI[2], G.-J. WANG[2], N. D. VOLKOW[2]; [1]Lab. of Neuroimaging, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; [2]NIAAA, Bethesda, MD; [3]NIAAA, Bethseda, MD

Abstract: Sleep disturbances and substance use disorders are highly comorbid conditions, but the neural mechanisms underlying this comorbidity remain largely unknown. It has consistently been found that cocaine abusers have reduced striatal dopamine D2 receptor (D2R) availability compared to controls. Moreover, it was recently shown that direct sleep deprivation decreases striatal D2R availability in healthy volunteers. In this study, we therefore investigated whether durations of daily sleep are associated with DR2 availability in a group of 24 active cocaine abusers and 21 healthy controls (matched for age [mean cocaine abusers =44.78years ± 4.88SD; mean controls = 42.18 years ± 5.49SD), sex, education, BMI, handedness and ethnicity) using Positron Emission Tomography (PET) and [11C]raclopride. We found that compared to controls, cocaine abusers had shorter sleep durations (mean cocaine abusers = 6.17h ± 1.61SD; mean controls = 7.43h ± 1.12SD; t=3.01, p=.004), went to bed later (mean cocaine abusers = 00:14 am ± 1.6h SD; mean controls = 11:14pm ± 1.51h SD; t= 2.26, p=.029) and reported longer periods of sleep disturbances (mean cocaine abusers = 19min ± 42min SD, mean controls = 0; t=2.33, p=.029), but did not feel less rested in the morning (cocaine abusers: 19 yes/5 no, controls: 18 yes/3 no χ^2=.33, p>.1). Further, cocaine abusers showed lower D2R availability in striatal areas than controls (putamen [p=.021] and ventral striatum [p.1]). In cocaine abusers, duration of sleep predicted D2R availability in all striatal areas (putamen [r^2=.218, p=.021], ventral striatum [r^2=.209, p=.025], and caudate [r^2=.276, p=.008]), also when correcting for age (all p.1). Moreover, in cocaine abusers, last cocaine use correlated with duration of sleep (cocaine use in mg [r=-.495, p=.014] and in $ [r=-.543, p=.006]), but not with striatal D2R availability (all p>.1). These findings suggest that cocaine-induced sleep deprivation reduces striatal D2R availability. Alternatively, shorter sleep and lower D2R may be risk factors for cocaine abuse.

Disclosures: C.E. Wiers: None. **E. Shumay:** None. **E. Cabrera:** None. **E. Skarda:** None. **E. Shokri-Kojori:** None. **S. Cunningham:** None. **C. Wong:** None. **D. Tomasi:** None. **G. Wang:** None. **N.D. Volkow:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.01/L43

Topic: C.17. Drugs of Abuse and Addiction

Support: National Basic Research Program of China (2015CB553504)

Nature Science Foundation of China (U1132602; 81471350)

Title: Optogenetic activation of anterior cingulate cortex inhibits the heroin seeking behavior in rats

Authors: *W. ZHOU, M. LAI, H. ZHU, W. CHEN, H. LIU, F. ZHANG;
Ningbo Addiction Res. and Treat. Cent. Med. Sch. of Ningbo Univ., Ningbo, China

Abstract: The aims of present study were to investigate effects of optogenetic activation of anterior cingulate cortex (ACC) on heroin self-administration, heroin seeking behavior induced by cues or heroin priming and learning and memory in rats. The rats in ACC optogenetic activation group were microinjected with 1μl pAAV-CaMKIIα-hChR2-EYFP into ACC(AP:+2.7mm,ML:0.5mm,DV:2.5mm), then implanted optical fiber 0.5mm above site of microinjection. The rats in sham operation group were microinjected with AVV-EYFP into ACC. The rats were placed in operant chambers for a daily 4h heroin self-administration session under FR1 for consecutive 14 days. Then memory was taken eight-arms maze test. Optogenetic activation of ACC by illuminated by light(470 nm,5mW) decreased the enhancement of reference memory errors, working memory errors induced by heroin exposure. Moreover, optogenetic activation of ACC inhibited the heroin seeking behavior induced cues, which could be reversed partially by pretreatment with LY341495. While, Optogenetic activation of NAc core(AP:+1.2mm,ML:±3.2mm,DV:-6.7mm)also inhibited the heroin seeking induced by cues. In conclusion, these data demonstrate that activation of ACC can improve the cognitive dysfunction induced by heroin chronic exposure, and projective pathway from ACC to NAc core may be involved in the heroin seeking behavior induced by cues.

Disclosures: W. Zhou: None. **M. Lai:** None. **H. Zhu:** None. **W. Chen:** None. **H. Liu:** None. **F. Zhang:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.02/L44

Topic: C.17. Drugs of Abuse and Addiction

Support: National Basic Research Program of China (2015CB553504)

Nature Science Foundation of China (U1132602; 81471350; 81171257)

Natural Science Foundation of Zhejiang Province (LY14H310002)

Ningbo Social Development Research Project (2013C50033).

Title: Role of deep brain stimulation of medial prefrontal cortex on heroin seeking behavior in rats

Authors: *H. LIU[1,2];
[1]Ningbo Inst. of Microcirculation and Henbane,Ningbo Addiction Res. and T, Zhejiang, China;
[2]Lab. of Behavioral Neuroscience, Sch. of Medicine, Ningbo Univ., Ningbo, China

Abstract: Recent research in both animals and humans has indicated that deep brain stimulation (DBS) may have potential for the treatment of drug dependence and relapse, but few studies of DBS on the treatment of heroin dependence, in particular for relapse to heroin seeking, it is not yet entirely clear which brain and paradigm should be applied. In the present study, we used an animal model of heroin self-administration to investigate the role and possible mechanisms of DBS treatment for the two sub-regions (dorsal and ventral) of medial prefrontal cortex (mPFC) in the extinction and reinstatement of heroin seeking behavior. Cue-induced heroin seeking reinstatement test was conducted 24h after the last extinction training. In the DBS treatment with dmPFC, two-factor repeated ANOVA revealed that the H-DBS group had significantly lower active pokes in cue-induced reinstatement of heroin seeking compared with those of sham stimulation group, while there was no difference in active pokes between sham stimulation and L-DBS group in cue-induced reinstatement of heroin seeking; There was also no difference in active nose-pokes between stimulated and sham control groups during the extinction sessions. In the DBS treatment with vmPFC, two-factor repeated ANONA found the number of active pokes in H-DBS group were significantly higher than the sham control group in cue-induced reinstatement of heroin seeking or during day 4-7 extinction sessions. We also explored the effects of long-term high frequency stimulation of dmPFC on p-CREB ,p-AKT and p-ERK expression in the NAc core and NAc shell. Western blot and immunohistochemical analysis showed that the level of p-CREB in the NAc core significantly increased in the rats treated with H-DBS compared with the sham control. In contrast, the level of p-ERK and p-AKt in the NAc core significantly decreased in the rats treated with H-DBS compared with the sham control. We conclude that the high frequency stimulation of dmPFC can inhibit the reinstatement of heroin-seeking induced by conditioned cues, and its regulation of phosphorylated CREB, phosphorylated ERK and phosphorylated AKt expression in NAc core may contribute to the behavioral inhibition in cue-induced reinstatement. High frequency stimulation of the vmPFC can impair the extinction of heroin seeking and facilitate the reinstatement of heroin-seeking induced by conditioned cues ; Low frequency stimulation of either dmPFC or vmPFC have no influence on heroin seeking behavior during extinction and cue-induced reinstatement. The present studies demonstrated that chronic DBS treatment with mPFC may represent a useful treatment method for heroin addiction.

Disclosures: H. Liu: None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.03/M1

Topic: B.05. Transporters

Support: NIH RO1 AA020610-01

Title: AMPA/Kainate receptor modulation of glutamate transporters in the prefrontal-accumbens circuitry: impact of adolescent ethanol drinking

Authors: *G. E. SEALE[1], T. MERCED[2], E. P. RODRIGUEZ[2], L. ACOSTA[2], R. I. MELENDEZ[2];
[1]Inst. of Neurobio., Univ. of Puerto Rico, San Juan, PR; [2]Anat. and Neurobio., Univ. of Puerto Rico, San Juan, Puerto Rico

Abstract: Glutamate transporters play an indispensable role in shaping synaptic glutamate transmission and plasticity. Alterations in the re-uptake of glutamate has been linked to a number of neurobiological disorders, including drug and alcohol addiction. Yet, the regulation of glutamate uptake systems by glutamatergic receptors remains not well understood. Here we examined the role of AMPA and kainate glutamate receptors in regulating glutamate uptake in the prefrontal cortex (PFC) and nucleus accumbens (NAC) of ethanol (EtOH) naïve and EtOH-drinking adolescent and adult C57BL/6J mice (n=4-6/group/age/drug). Mice were initially given 24 h two-bottle choice access to 15% EtOH and water for 2 weeks, which spans the adolescent period. Mean EtOH intake prior to glutamate uptake procedures was (in g/kg) 12.3 ± 0.6 for adolescent and 11.1 ± 0.5 for adult mice. Following EtOH drinking, crude (mixed) synaptosomal preparations were obtained from the PFC and NAC of all mice and subjected to a [3H]-glutamate uptake assay. In naïve mice, our results indicated that the maximal velocity or Vmax of glutamate uptake was significantly greater in PFC than NAC of both age groups, suggesting a greater number of glutamate transporters in PFC. The Vmax for glutamate uptake was (in pmol/mg protein/min) 18.1 ± 0.7 in PFC and 13.4 ± 1.2 in NAC, respectively. Interestingly, in both age groups, we revealed a significant (nearly 30%) enhancement of glutamate uptake following application of the AMPA/kainate antagonist, DNQX (50 μM) in NAC but not PFC. On the other hand, application of the AMPA-selective antagonist NBQX (50 μM) significantly reduced (nearly 50%) glutamate uptake in NAC particularly in adult mice, but not in PFC. In EtOH mice, we observed a significant reduction in the Vmax of basal glutamate uptake in NAC but not PFC, particularly in adolescent mice. Moreover, EtOH drinking experience significantly blocked the ability of DNQX to stimulate glutamate uptake in the NAC in both age groups, and further enhanced the ability of NBQX to inhibit NAC glutamate uptake, particularly in

adolescent mice. These findings indicate that AMPA and kainate receptors differentially modulate glutamate transporters particularly in the NAC of B6 mice. Moreover, EtOH drinking experience blocks DNQX-induced potentiation and enhances NBQX-induced inhibition of glutamate uptake, which may lead to major deficits in synaptic glutamate transmission and plasticity.

Disclosures: **G.E. Seale:** None. **T. Merced:** None. **E.P. Rodriguez:** None. **L. Acosta:** None. **R.I. Melendez:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.04/M2

Topic: C.17. Drugs of Abuse and Addiction

Support: PIP 11420100100072, Argentina

PICT 2012-0924, Argentina

PICT-2012-1769, Argentina

UBACYT 2014-2017 #20120130101305BA, Argentina

NIH award P20 GM103425 to UAMS, USA

Title: Epigenetic alterations in medial prefrontal cortex and testis after chronic caffeine and cocaine administration in mice

Authors: *B. GONZALEZ[1], C. R. GONZALEZ[2], J. A. MUÑIZ[1], J.-L. CADET[3], E. GARCIA-RILL[4], F. URBANO[5], A. VITULLO[2], V. BISAGNO[1];
[1]ININFA - Pharmacol. Res. Inst., Buenos Aires, Argentina; [2]Ctr. de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD),, Univ. Maimónides, Buenos Aires CABA, Argentina; [3]Natl. Inst. on Drug Abuse, Baltimore, MD; [4]Univ. of Arkansas for Med. Sci., Little Rock, AR; [5]IFIByNE-CONICET, Buenos Aires, Argentina

Abstract: There is growing evidence of epigenetic mechanisms contributing to the transgenerational transmission of stress, psychiatric disease and addiction vulnerability. Regarding paternal transmission, it has been proposed that environmental factors like chronic stress, nutritional status, toxins and drugs of abuse trigger epigenetic mechanisms in the testicular germ line that can lead to variations in offspring's development and behavior. Chronic psychostimulant intake also causes epigenetic changes and toxic consequences not only in dopaminergic brain areas but in peripheral organs as well, including the testis. From that perspective, several groups have reported epigenetic alterations in the medial prefrontal cortex

(mPFC) of cocaine-experienced sires that were present in the testicular germ line and were transmitted to the male offspring. Recreational use of cocaine (Coc) is often abused in combination with other psychostimulants like caffeine (Caf). Caf was reported to enhance cocaine-mediated effects in brain areas. In the present study, we measured epigenetic and functional markers in the mPFC and testis of adult mice treated with Coc (10 mg/kg), Caf (5 mg/kg), or the combination (Caf-Coc) (10 mg/kg Coc + 5 mg/kg Caf) compared to vehicle (Veh), in an intermittent binge protocol (3 i.p. injections, 1 h apart, one day on/off for 13 days. Mice were euthanized on day 14. Deacetylation by histone deacetylases (HDACs) at lysine residues are post-translational histone modifications that have been reported in various neuropsychiatric diseases including addiction. In our study, we found that Coc and Caf-Coc treatments caused a decrease of HDAC2 expression and a concomitant increase in histone 3 acetylation (H3ac) in both mPFC and testis. Morphometric analyses of the testis revealed reduced volume of the seminiferous tubules after Coc and Caf-Coc treatments, indicative of altered spermatogenic process. In addition, there was reduced mRNA expression of AMPA-type glutamate receptor subunit Gria1, adenosine receptor subunit Adora2a, scaffolding protein Psd95 and methyl CpG binding protein Mecp2 in the mPFC after Caf, Coc and Caf-Coc treatments. We are currently investigating whether these psychostimulants might differentially regulate gene expression in the testis. Our results further support that psychostimulant abuse can induce epigenetic marks in CNS and testis that could lead to impaired function with the possibility of affecting individuals from next generation.

Disclosures: **B. Gonzalez:** None. **C.R. Gonzalez:** None. **J.A. Muñiz:** None. **J. Cadet:** None. **E. Garcia-Rill:** None. **F. Urbano:** None. **A. Vitullo:** None. **V. Bisagno:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.05/M3

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: P50 AA010761

Title: Monoamines alter neuronal excitability of lateral orbitofrontal cortex neurons: role of G protein-coupled inwardly-rectifying potassium channels

Authors: ***S. NIMITVILAI**, M. F. LOPEZ, P. J. MULHOLLAND, J. J. WOODWARD; Dept. of Neurosci. and Addiction Sci. Div., Med. Univ. of South Carolina, Charleston, SC

Abstract: The orbitofrontal cortex (OFC), a brain region within the prefrontal cortex, plays an important role in integrating sensory information, and participates in learning, prediction and decision making for emotional and reward-related behaviors. Many studies have revealed that

the OFC is extensively innervated by monoamines, including serotonin, dopamine and noradrenaline. Dysfunction of the OFC is associated with numerous neuropsychiatric disorders such as schizophrenia, obsessive-compulsive disorder as well as alcohol and substance abuse disorders, and drugs that target monoamine receptors have been used in the treatment of these psychiatric diseases. With whole-cell patch-clamp electrophysiology, we found that 5HT, DA or NE produced a dose-dependent decrease in spike firing of lateral OFC (lOFC) neurons. Pharmacological studies revealed that these effects were mediated via Giα-coupled 5HT1A, D2 or α2-adrenergic receptors, respectively. As activation of these and other Giα-coupled receptors has been linked to stimulation of G protein-coupled inwardly rectifying potassium (GIRK) channel, we examined whether the GIRK channel is a common target for monoamine-induced inhibition of spiking. In addition, we tested whether acute and chronic ethanol exposure (CIE) altered the spiking when GIRK channels were modulated. Inhibition of neuronal firing by 5HT, DA or NE was suppressed by a GIRK channel blocker barium, while the GIRK channel activator ML297 decreased firing when administered alone. In the presence of ML297 or barium, acute ethanol (33 mM) had no further effect on firing. In neurons from mice exposed to CIE, spike frequency was nearly doubled and these neurons were largely resistant to the inhibitory effects of acute ethanol or each monoamine for up to at least 7 days of withdrawal. ML297 also had little effect on spiking in neurons from CIE-treated animals, despite no change in GIRK channel expression. Since activation of GABA-B receptor is also known to stimulate GIRK channels, we tested whether the GABA-B receptor agonist baclofen affects lOFC excitability. In control animals, baclofen decreased spiking of lOFC neurons, and this inhibition was also diminished in neurons obtained from CIE-exposed mice. The results of these studies suggest that monoamines likely play a key role in modulating the intrinsic excitability of lOFC neurons, through activation of GIRK channels, and that this modulation is significantly disrupted following chronic exposure to alcohol. Dysfunction of one or more of these neuromodulators may contribute to impaired OFC function associated with various neuropsychiatric diseases including alcohol dependence.

Disclosures: **S. Nimitvilai:** None. **M.F. Lopez:** None. **P.J. Mulholland:** None. **J.J. Woodward:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.06/M4

Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC Grant 92057

CIHR Grant 137122

NIH Grants DAO32933, DAO33760

Title: Pharmacological inhibition of monoacylglycerol lipase systemically and centrally in the amygdala and visceral insular cortex prevents establishment of a naloxone-precipitated morphine induced conditioned place aversion in rats

Authors: *K. L. WILLS[1], C. L. LIMEBEER[1], E. M. ROCK[1], M. J. NIPHAKIS[2], B. F. CRAVATT[2], L. A. PARKER[1];
[1]Univ. of Guelph, Guelph, ON, Canada; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: Enhancement of endocannabinoid activity, through pharmacological inhibition of the catabolic enzyme fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL), has been shown to reduce somatic symptoms of morphine withdrawal in mice (Ramesh et al., 2011; Ramesh et al., 2013). However, the effect of such treatments on the affective properties of morphine withdrawal has not been investigated in rats. The conditioned place aversion paradigm represents an animal model capable of assessing the ability of pharmacological manipulations to alter affective morphine withdrawal. Specifically, a morphine induced conditioned place aversion is produced when naloxone is administered 24 hr following a single exposure to a high dose of morphine (Parker and Joshi, 1998). Our experiments demonstrate that systemic pretreatment with the monoacylglycerol lipase (MAGL) inhibitor, MJN110 (which selectively elevates the endocannabinoid 2-arachidonoylglycerol - 2-AG), but not the fatty acid amide hydrolase (FAAH) inhibitors, URB-597 and PF-3845 (which selectively elevate the endocannabinoid anandamide - AEA), interferes with the establishment of a naloxone-precipitated conditioned place aversion; a model of affective morphine withdrawal. Furthermore, central administration of MJN110 to regions of the amygdala or to the visceral insular cortex (VIC) also prevents the establishment of the place aversion. The effect of MJN110 to interfere with withdrawal was reversed with pretreatment of the CB1 antagonist AM251, and MJN110 administration alone did not possess rewarding or aversive properties in the place conditioning paradigm. Ultimately, these findings suggest pharmacological treatments that elevate 2-AG acting at the CB1 receptor may be useful in reducing the aversive effects of morphine withdrawal.

Disclosures: K.L. Wills: None. **C.L. Limebeer:** None. **E.M. Rock:** None. **M.J. Niphakis:** None. **B.F. Cravatt:** None. **L.A. Parker:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.07/M5

Topic: C.17. Drugs of Abuse and Addiction

Title: Alterations in sleep architecture of older people users of psychoactive substances

Authors: *M. M. MELENDEZ[1,2], N. HERNANDEZ[1], A. GALLEGOS-CARI[1], S. MUÑOZ-SANCHEZ[1], R. CAMACHO-SOLÍS[1], F. AYALA-GUERRERO[3], U. JIMENEZ-CORREA[4], A. JIMENEZ-ANGUIANO[2];
[1]IAPA-DF, Mexico City, Mexico; [2]Área de Neurociencias, Dept. de Biología de la Reproducción, Univ. Autónoma Metropolitana-Iztapalapa, México DF, México City, Mexico; [3]Univ. Nacional Autónoma de México, Facultad de Psicología. Área de Neurociencias, Mexico City, Mexico; [4]Univ. Nacional Autónoma de México, Facultad de Medicina, Clínica de Trastornos de Sueño, Mexico City, Mexico

Abstract: Introduction: Sleep disturbances are related with increased risk of depression, hearth diseases, cognitive decline and primary sleep disorders in elderly [1]. About 15 to 45% of older adults in the US reported difficult of initiate and maintain sleep [2] and in Mexico City about 63.2% of older people reports bad sleep quality [3]. Many risk factors are related to sleep disturbances in the elder [4]. It is also known that alcohol [5] and drug consumption [6] produces changes in sleep architecture, suppressing REM sleep and increasing arousal, but little is now about the relationship between aging, sleep disturbances and drug consumption. Method: A descriptive, retrospective, correlational study was performed. A methodological review of 148 files of patients who attended to a Sleep Clinic was done. Clinical history and polisomnographic report was analyzed, to assess differences between history of alcohol and drug consumption and polysomnographic variables. Descriptive statistics of the group were determined. Four groups were studied (caffeine use, alcohol use, polydrug use and no substance use). Means were compared between groups using ANOVA with DMS post-hoc test, and for robust mean comparison used Welch's and Brown-Forsythe statistic. Results: Mean age was 70 years old; we found differences in S1% (W=5.690, sig=.001), Apnea/Hypoapnea Index (W=6.454, sig<.001), O2% Total (B-F=3.391, sig=.014), and post-hoc analysis showed significant differences in alcohol consumption in Arousal%, caffeine in REM latency, caffeine in Total O2%, polydrug use in Minimum O2% and caffeine in apnea index. References 1.- Vaz Fragoso CA & Gill TM. (2007). Sleep complaints in community-Living older persons: a multifactorial geriatric syndrome. J Am Geriatr Soc; 55(11): 1853-1866. 2.- Ohayon MM.(2002). Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev; 6:97-111. 3.- Mendoza-Melendez MA, Hernandez-Llanes NF, Gallegos-Cari A, Jimenez-Correa U, Ayala-Guerrero F, Velázquez-Moctezuma J, Jimenez-Anguiano A, Sanchez-Sosa JJ, Borges G & Medina-Mora MA. (en prensa). Association between sleep quality and hazardous alcohol drinking elderly in Mexico City. Sleep. 4.- Adib-Hajbaghery M, Izadi-Avanji F & Akbari H. (2012). Quality of sleep and its related risk factors in hospitalized older patients in Kashan's Hospitals, Iran 2009. Iran J Nurs Midwifery Res, 17(6): 414-420. 5.- NIAA. (1998). Alcohol and sleep. Alcohol Alert; 41. 6.- Schierenbeck T, Riemann D, Berger M & Hornyak M. (2008). Effect of illicit recreational drugs upon sleep: Cocaine, ecstasy and marijuana. Sleep Medicine Reviews; 12: 381-389.

Disclosures: M.M. Melendez: None. **N. Hernandez:** None. **A. Gallegos-Cari:** None. **S. Muñoz-Sanchez:** None. **R. Camacho-Solís:** None. **F. Ayala-Guerrero:** None. **U. Jimenez-Correa:** None. **A. Jimenez-Anguiano:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.08/M6

Topic: C.17. Drugs of Abuse and Addiction

Support: This research was supported [in part] by the Intramural Research Program of the NIH, NIDA

Title: Distinct neuronal ensembles in rat infralimbic cortex control food reward memories and extinction memories

Authors: *B. L. WARREN, M. P. MENDOZA, F. C. CRUZ, R. M. LEAO, D. CAPRIOLI, K. B. MCPHERSON, Y. SHAHAM, B. T. HOPE;
NIDA IRP/NIH, Baltimore, MD

Abstract: Animals can be trained to perform an operant response to receive a reward and then to extinguish the learned response when the reward is withheld. Reward memories and extinction memories are thought to be distinct and are likely encoded by different patterns of sparsely distributed neurons called ‘neuronal ensembles’. In a previous study, we found elevated Fos immunoreactivity in the infralimbic cortex in rats exposed to 2 days of extinction training, suggesting that neuronal ensembles within the infralimbic cortex encode extinction memory. To test this hypothesis, we first trained food-restricted transgenic cfos-lacZ rats to lever press for palatable food pellets for 7 days (60 min/day). Two groups of rats were subsequently exposed or not exposed (levers retracted) to 2 days of extinction training (60 min/day). On induction day, one day later, all rats were exposed to a brief induction session (15 min) under extinction conditions to induce Fos. After 75 additional minutes, we selectively inactivated infralimbic neuronal ensembles associated with either the food reward memory (no extinction group) or the extinction memory (extinction group) using the Daun02 inactivation procedure to determine the effects of inactivating ensembles on food seeking. We hypothesized that the ‘food reward ensemble’ is reactivated on induction day when there was no prior extinction training and that the ‘extinction ensemble’ is reactivated after 2 days of prior extinction training. Two days after Daun02 inactivation, rats in the no extinction group had decreased, while rats in the extinction group had increased their lever presses in a brief (15 min) test under extinction conditions. Here, we show that selective inactivation of extinction ensembles impaired extinction recall, while selective inactivation of reward ensembles disrupted reward recall. Results demonstrate that neuronal ensembles encoding reward and extinction memories can intermingle in the same brain area. This research was supported [in part] by the Intramural Research Program of the NIH, NIDA

Disclosures: B.L. Warren: None. **M.P. Mendoza:** None. **F.C. Cruz:** None. **R.M. Leao:** None. **D. Caprioli:** None. **K.B. McPherson:** None. **Y. Shaham:** None. **B.T. Hope:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.09/M7

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA IRP / NIH

Title: Fos-expressing neuronal ensembles in learned behaviors using the Fos-Tet-Cre transgenic rat system

Authors: *R. MADANGOPAL, B. L. WARREN, D. CAPRIOLI, B. LIANG, L. R. WHITAKER, R. M. LEAO, F. CRUZ, F. J. RUBIO, Y. ZHANG, C. T. RICHIE, D.-T. LIN, Y. SHAHAM, B. K. HARVEY, B. T. HOPE;
Natl. Inst. On Drug Abuse IRP, Baltimore, MD

Abstract: Since proposed by Hebb in 1949, evidence supporting the hypothesis that learned associations are encoded in sparsely distributed 'cell assemblies' (neuronal ensembles) has primarily been based on correlations between *in vivo* electrophysiological firing or two-photon calcium-imaging patterns during learning and memory tasks and learning-related post-mortem activity patterns of immediate early genes (IEGs) such as c-fos, arc and zif268. Until recently, the lack of ensemble-targeted approaches has made it difficult to examine whether this sub-population of neurons that is selectively activated during learned behaviors mediates these associations. Current methods target all neurons within a specific region or belonging to a specific cell-type, regardless of whether or not they were selectively activated during learned behaviors. Recently, the Hope lab developed a novel tool_the Fos-Tet-Cre transgenic rat system_that permits recombination of Cre-inducible genes in activated Fos-expressing neuronal ensembles within a 6-h time window following systemic tetracycline injections. The system was validated using a rat model of context-induced relapse to cocaine seeking using Cre-inducible YFP and Fos-immunoreactivity to assess context-specific reactivation of neuronal ensembles in ventral mPFC. Further, selective inhibition of these context-encoding neuronal ensembles using a Cre-inducible halorhodopsin decreased context-induced relapse. We are now pursuing high resolution mapping of neural circuit dynamics in freely behaving Fos-Tet-Cre transgenic rats using a combination of GRIN endoscope-based calcium imaging, two-photon microscopy, and viruses for cell-type and task specific expression of fluorescent reporter proteins.

Disclosures: R. Madangopal: None. **B.L. Warren:** None. **D. Caprioli:** None. **B. Liang:** None. **L.R. Whitaker:** None. **R.M. Leao:** None. **F. Cruz:** None. **F.J. Rubio:** None. **Y. Zhang:** None. **C.T. Richie:** None. **D. Lin:** None. **Y. Shaham:** None. **B.K. Harvey:** None. **B.T. Hope:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.10/M8

Topic: C.17. Drugs of Abuse and Addiction

Support: Chang Gung Memorial Hospital at Keelung Branch, Taiwan: CMRPD1D0291

Title: The neural substrates underlying ketamine addiction

Authors: *I.-M. LIAO[1], J.-C. CHEN[2];
[2]Dept. of Physiol. and Pharmacol., [1]Grad. Inst. of Biomed. Sciences, Chang Gung Univ., Taoyuan City, Taiwan

Abstract: Ketamine (KET), an NMDA receptor antagonist, displays a diverse pharmacological profile, in which the drug effects are differentiated based on the dosage used. High dose of KET leads to anesthesia, sub-threshold dose exhibits anti-depressant effect, while as sub-anesthesia dose with chronic consumption induces addiction, as well as symptoms that model for schizophrenia or KET psychosis. The action of anti-depressant effect of KET has been explored extensively in the recent years; however, the cellular mechanism of KET addiction has been rarely addressed. The specific aim of this study is to explore the neural substrates of KET addiction and its rewarding properties. In the present study, mice receiving daily 30 mg/kg intraperitoneal KET administration for 7 consecutive days developed locomotor sensitization. Dopamine and glutamatergic signaling cascades implicated in addiction progress were assessed accordingly in the nucleus accumbens (NAc). Increased phosphorylation of protein kinase B (PKB/Akt) and glycogen synthase kinase (GSK3) were observed in the NAc in mice after 7 days of KET treatment. In addition, the phosphorylation of extracellular signal-regulated kinase (ERK) 2 and AMPA receptor at GluR1 were also upregulated in the NAc after 7 days of KET treatment. These results imply the potential action of KET in modulating and converging both dopaminergic and glutamatergic neural transmission in the NAc at a sub-anesthesia dose. The enhanced GluR1 phosphorylation may represent a possible long-term potentiation (LTP) in accumbal synaptic strength contributing to addiction learning. The functional aspect of these signal changes in KET sensitization is currently under investigation to elucidate the neuroplasticity underlying KET addiction.

Disclosures: I. Liao: A. Employment/Salary (full or part-time):; Chang Gung University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Chang Gung Memorial Hospital at Keelung Branch. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Chang Gung University. **J. Chen:** A. Employment/Salary (full or part-time):; Chang Gung University. B. Contracted

Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Chang Gung Memorial Hospital at Keelung Branch. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Chang Gung University.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.11/M9

Topic: C.17. Drugs of Abuse and Addiction

Support: PPF 2015 (14I067.01/1)

PSI 2011-29181

UJI (14I307.01/1)

Title: Expression of Perineuronal Nets in the cerebellum after Prelimbic lesions in rats trained to acquire conditioned preference towards cocaine-related cues

Authors: *I. GIL MIRAVET[1,2], M. CARBO-GAS[1], C. SANCHIS-SEGURA[1], D. CARULLI[2], M. MIQUEL[1];
[1]Psychobiology, Univ. Jaume I, Castellon, Spain; [2]Dept. of Neurosci. Rita Levi-Montalcini. NICO, Universiti of Turin, Turin, Italy

Abstract: Pavlovian conditioning tunes the motivational drive for drug-associated stimuli, fostering the probability of those environmental stimuli to promote and trigger drug seeking and taking. The permanent capability of these cues to trigger drug consumption derives from an over-consolidation of such drug-dependent Pavlovian memories. Growing evidence supports that reorganization of the prefronto-striatal-limbic networks underpins storage of these drug-induced memories. For years, the cerebellum has been a neglected region in brain circuits holding these long-lasting drug-related memories. This is surprising because several decades of research have demonstrated that prefrontal-cerebellar loops are strongly compromised in drug addiction. Moreover, clinical data suggest that lesions and pathological conditions reorganize functions of the prefrontal-cerebellar circuitry. Recently, we have found two cerebellar hallmark signatures of conditioned preference for cocaine: an increase in cFOS expression in cells at the apex of the granule cell layer as well as strong expression of the perineuronal nets (PNNs) surrounding Golgi interneurons at the same region of the cerebellar vermis. No one of these cerebellar features was seen if mice did not develop preference for the cocaine-related cue. The present study aimed to evaluate the effects of prelimbic deactivations on the acquisition of cocaine-induced conditioned preference as well as on the expression of PNNs in the cerebellar vermis. Rats were infused with

6% lidocaine (1microl/2min) ten minutes before conditioning. Remarkably, the results indicated that deactivation of the prelímbic cortex increases up to 100% the percentage of animals acquiring conditioned preference for cocaine as compared to the control group (sham). Golgi interneurons of the prelimbic lesion group expressed stronger intensity of wisteria floribunda agglutinin labelling (WFA) than the sham group, pointing to stronger PNNs. Our findings suggest that prelimbic lesions promote the acquisition of cocaine-induced cue-related memories and the development of those cerebellar hallmark signatures associated with such memories.

Disclosures: I. Gil Miravet: None. **M. Carbo-Gas:** None. **C. Sanchis-Segura:** None. **D. Carulli:** None. **M. Miquel:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.12/M10

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA R01 DA013951

Title: The effects of chronic adolescent toluene on behavioral flexibility and intrinsic excitability of mPFC neurons

Authors: *K. M. BRAUNSCHEIDEL[1], B. H. EATON[2], P. A. ZAMUDIO-BULCOCK[2], J. T. GASS[1], J. J. WOODWARD[1];
[1]Neurosci., [2]Psychiatry and Behavioral Sciences, Addiction Sci. Div., Med. Univ. of South Carolina, Charleston, SC

Abstract: Psychoactive inhalants such as toluene induce changes in neural connectivity, electrophysiology, and behavior that are similar to those produced by classic drugs of abuse. Toluene and other abused inhalants are predominantly used by adolescents, and exposure to these compounds may contribute to behavioral problems associated with drug use such as compulsivity and reduced executive control. While use of volatile solvents is associated with deficits in fronto-cortical dependent behaviors in humans, studies of solvents using well-validated animal models of behavioral flexibility are lacking. To address this issue, we treated male, adolescent Sprague-Dawley rats (P39 at first exposure) to ten minute, twice daily exposures to toluene (~5400PPM) for five consecutive days. After reaching adulthood (P60) rats were trained in operant boxes to lever-press in response to a light cue for 20% sweetened condensed milk. We then tested behavioral flexibility using an attentional set-shifting task followed by a reversal learning task. As predicted, rats that experienced chronic toluene exposure during adolescence took longer to learn the initial reward contingencies during training. Surprisingly however, the toluene exposed group reached criteria in significantly fewer trials

compared to air treated controls during the set-shifting task, a result driven by a marked reduction in perseverative errors. There were no significant differences between the two groups during the reversal learning task. Lesion and pharmacological studies have identified two subdivisions of the frontal cortex, the medial prefrontal cortex (mPFC) and orbital frontal cortex (OFC), as regions responsible for attentional set-shifting and reversal learning, respectively. We thus hypothesized that the behavioral alterations observed in toluene treated rats could be due to altered mPFC neuron electrophysiology and dendritic spine morphology. To test this idea, we performed current-clamp electrophysiology and spine imaging on separate cohorts of adult animals treated with toluene vapor during adolescence. To date, results from electrophysiology experiments reveal that chronic adolescent toluene exposure enhances measures of excitability including a hyperpolarized action potential threshold and reduced after hyperpolarization potential. These data suggest that toluene selectively alters mPFC-dependent behavior, which could be a result of enhanced neuronal excitability in this region. Understanding the effects of chronic adolescent toluene could provide further insight into the neural basis of both drug addiction and behavioral flexibility Supported by NIDA R01 DA013951.

Disclosures: K.M. Braunscheidel: None. **B.H. Eaton:** None. **P.A. Zamudio-Bulcock:** None. **J.T. Gass:** None. **J.J. Woodward:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.13/M11

Topic: C.17. Drugs of Abuse and Addiction

Support: Centre National de la Recherche Scientifique (CNRS)

Agence Nationale de la Recherche (grant ANR- 2010-BLAN-1404-01)

Université de Bordeaux

Conseil Regional d'Aquitaine (grants CRA11004375 and CRA11004699)

Fondation NRJ

Title: Neuronal activity in the orbitofrontal cortex predicts cocaine choices and preferences in addicted rats

Authors: *K. GUILLEM[1,2], S. H. AHMED[1,2];
[1]Univ. De Bordeaux, Bordeaux Cedex, France; [2]CNRS, Bordeaux Cedex, France

Abstract: Human neuroimaging research has consistently shown that cocaine addiction is associated with structural and functional changes within the orbitofrontal cortex (OFC). In view

of the important role of the OFC in value-based decision-making, these changes have been hypothesised to bias choice towards cocaine despite and at the expense of other competing pursuits, thereby explaining cocaine addiction. Here we report for the first time direct evidence for this hypothesis in a choice-based model of cocaine addiction where rats could choose between two actions, one rewarded by cocaine, the other by a nondrug alternative. Briefly, we used *in vivo* electrophysiology to record the neuronal correlates of individual choices and preferences in a minority of cocaine-preferring rats versus a majority of non-drug preferring rats. We first assessed action-coding selectivity of OFC neurons and then followed their firing activity before and during choice. We found that the relative proportion of OFC neurons encoding the cocaine-rewarded action versus the alternative action matched individual preferences. Specifically, in cocaine-preferring rats, the proportion of neurons encoding the cocaine-rewarded action was larger than that encoding the alternative action (38 versus 31%), while it was the opposite in nondrug-preferring rats (22 versus 53%). Moreover, pre-choice relative firing activity of both functional populations of neurons predicted several seconds in advance what animals will eventually prefer. Finally, to assess causality, we conducted a pharmacological intervention known to shift choices toward cocaine in the majority of nondrug-preferring rats. This induced reversal in preference was associated with a dramatic reversal of the predictive neuronal correlates of preference, mostly due to an overall and relatively selective suppression of firing activity in OFC neurons encoding the alternative action. Overall, this study demonstrates that OFC neurons play a critical role in influencing cocaine choices and preferences in addicted rats.

Disclosures: K. Guillem: None. **S.H. Ahmed:** None.

Poster

321. Cortical Plasticity in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 321.14/M12

Topic: B.08. Synaptic Plasticity

Support: University of Bristol Alumni Scholarship SOCS NE1206

Title: New street drugs block NMDA receptor mediated synaptic excitation

Authors: *H. KANG[1], Z. A. BORTOLOTTO[2], S. LIGHTMAN[2], G. L. COLLINGRIDGE[2], J. WALLACH[3], S. D. BRANDT[3], D. LODGE[2];
[1]Med. and Dent., [2]Univ. of Bristol, Bristol, United Kingdom; [3]Liverpool John Moores Univ., Liverpool, United Kingdom

Abstract: Dissociative anaesthetics, such as ketamine and phencyclidine, induce a range of psychoactive effects in humans, and have been widely used as street drugs for recreational purposes. Since these drugs have been placed under legislative control, new designer drugs

related to diphenidine have appeared and have been associated with similar perceptual effects. In addition, intoxication has been reported to produce hypertension, confusion and psychosis as well as casualties. Beside diphenidine (DPH) itself, several derivatives including the 2-chloro (2-Cl-DPH) and 3-methoxy (3-MeO-DPH) are known and may, like ketamine, have N-methyl-D-aspartate (NMDA) receptor antagonist activity. To investigate the potential NMDA receptor antagonist properties of these three new street drugs, we have compared their actions with those of standard channel blockers, ketamine, memantine and dizocilpine (MK-801). Using adult rat hippocampal slices, isolated NMDA receptor-mediated field excitatory postsynaptic potentials (NMDAR-fEPSPs) were recorded from CA1 neurons following Schaffer collateral - commissural stimulation. DPH, 2-Cl-DPH and 3-MeO-DPH (all at 10 μM) like ketamine and memantine (10 μM) and MK-801 (0.3 μM) inhibited NMDAR-fEPSPs to a similar extent (70-90%). However, the time course of the inhibition was different. Ketamine reached a near plateau response in 2 h whereas fEPSPs decreased more slowly with all the other drugs, requiring some 5 h to reach near maximal effects. Interestingly, ketamine 1, 5 and 10 μM inhibited the NMDAR-fEPSP in a dose dependent manner, each concentration producing both a different rate of onset and a different maximum inhibition, whereas with 2-Cl-DPH, 1 μM the time course was notably longer than with 10 μM, requiring 10 hours but reached a similar maximal effect. In conclusion, these new DPH-related street drugs are effective blockers of NMDA receptor mediated synaptic transmission in the hippocampus.

Disclosures: H. Kang: None. **Z.A. Bortolotto:** None. **S. Lightman:** None. **G.L. Collingridge:** None. **J. Wallach:** None. **S.D. Brandt:** None. **D. Lodge:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.01/M13

Topic: C.17. Drugs of Abuse and Addiction

Support: R01HL105635

P50DA03615

Title: Menthol attenuates respiratory irritation responses to cigarette smoke and oral nicotine aversion in C57BL/6 mice: Role of TRPM8

Authors: *S. E. JORDT[1,2], S. BALAKRISHNA[2], L. FAN[2], A. I. CACERES[1], Y.-S. LIU[3], M. A. HA[4], G. J. SMITH[4], J. A. CICHOCKI[4], M. R. PICCIOTTO[2], J. B. MORRIS[4];
[1]Anesthesiol., Duke Univ., Durham, NC; [2]Tobacco Ctr. of Regulatory Science, Psychiatry, [3]Pharmacol., Yale Univ., New Haven, CT; [4]Pharmaceut. Sci., Univ. of Connecticut, Storrs, CT

Abstract: Addition of menthol to cigarettes may be associated with increased initiation of smoking, however, the mechanisms underlying this association are not known. Menthol, possibly through its effects on TRPM8 ion channels in cold-sensing peripheral sensory neurons, is known to inhibit the sensation of irritation elicited by respiratory irritants activating TRPA1, TRPV1 and other chemosensory irritant receptors. However, it remains unclear whether menthol modulates cigarette smoke irritancy and nicotine absorption during initial exposures to cigarettes, thereby facilitating smoking initiation. Using plethysmography in a C57Bl/6J mouse model, we examined the effects of L-menthol, the menthol isomer added to cigarettes, on the respiratory sensory irritation response to primary smoke irritants (acrolein and cyclohexanone) and smoke of reference cigarettes. We studied L-menthol's effect on blood levels of the nicotine metabolite, cotinine, immediately after exposure to cigarette smoke. We also examined the effects of menthol on oral nicotine aversion in mice in the two bottle drinking paradigm. L-menthol suppressed the irritation response to acrolein with an apparent IC50 of 4 ppm. Suppression was observed even at acrolein levels well above those necessary to produce a maximal response. Respiratory irritation caused by cigarette smoke was significantly suppressed by L-menthol even at smoke concentrations as high as 300 mg/m3. L-menthol's effects were abolished by treatment with a selective inhibitor of TRPM8, the neuronal cold/menthol receptor. Inclusion of menthol in the cigarette smoke resulted in a ~1.5-fold increase in plasma cotinine levels over those observed in mice exposed to smoke without added menthol. In the two bottle drinking paradigm addition of menthol reduced oral aversion to nicotine. This effect was reversed in Trpm8-deficient mice which showed an aversion to mentholated solutions. These findings document that, L-menthol, through TRPM8, is a strong suppressor of respiratory irritation responses, even during highly noxious exposures to cigarette smoke or smoke irritants, and increases blood cotinine. In addition to its effects on respiratory irritant sensing, menthol also suppressed aversion to oral nicotine, known for its irritating effects and bitter taste. The data suggest that L-menthol, as a cigarette additive, may promote smoking initiation and nicotine addiction.These effects may extend to other tobacco products, including electronic cigarettes and smokeless tobacco products.

Disclosures: **S.E. Jordt:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Amgen Pharmaceuticals. **S. Balakrishna:** None. **L. Fan:** None. **A.I. Caceres:** None. **Y. Liu:** None. **M.A. Ha:** None. **G.J. Smith:** None. **J.A. Cichocki:** None. **M.R. Picciotto:** None. **J.B. Morris:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.02/M14

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 5R01AA013588-14

Title: Protein Kinase C epsilon inhibitors as potential new therapeutics for alcohol use disorder

Authors: *A. BLASIO[1], D. WANG[2], R. O. MESSING[1,2];
[1]Univ. of Texas At Austin Col. of Pharm., Austin, TX; [2]Univ. of California, San Francisco, CA

Abstract: Although alcohol use disorder is a major public health problem worldwide, only three drugs have been approved by the FDA for treatment: Disulfuram, Naltrexone and Acamprosate. Our previous studies have provided strong evidence that the enzyme protein kinase C epsilon (PKCepsilon) promotes ethanol consumption and reward. We recently identified a lead compound derived from the commercially available Rho-associated coiled-coil forming protein kinase (ROCK) inhibitor Y-27632, which also inhibits PKCepsilon. This compound (named 12.0) and 3 analogs inhibited PKCepsilon with *K*i < 20 nM. All four compounds weakly inhibited conventional PKCgamma at 10 μM, while 2 compounds (12.3 and 12.7) showed almost no activity against atypical PKCzeta at that high concentration. All inhibited the highly related novel PKCs, PKCdelta and PKCteta, but showed some selectivity within the novel PKC subfamily being ~ 6 to 10-fold less potent in inhibiting PKCdelta and PKCteta than PKCepsilon. Two compounds (12.0 and 12.7) were screened against a panel of 395 non-mutant kinases using a binding competition assay (KINOMEscan™ Profiling Service from DiscoverRx) at a concentration of 200 nM. Both were potent and selective. Only twelve other kinases besides PKCepsilon were inhibited to <35% of control activity by these compounds and among these, only ROCK1 and ROCK2 were inhibited to less than 1% of control. A pharmacokinetic study with 12.0 (20 mg/kg) showed that it enters the brain and achieves a brain/plasma ratio of 0.53 at 4 hours, with a brain half-life of ~15.11 hours. We tested the ability of 2 compounds to reduce ethanol consumption in C57BL/6J mice using an intermittent access, two-bottle choice procedure. We administered the compounds using a within-subject, Latin square design (0, 10, 20 and 40mg/kg, *i.p.*). Compounds 12.0 and 12.3 dose-dependently reduced voluntary ethanol consumption. Other parameters such as water intake and food intake were not affected suggesting that 12.0 and 12.3 are well tolerated. Studies of conditioned place preference, taste preference and locomotor activity are ongoing.

Disclosures: **A. Blasio:** None. **D. Wang:** None. **R.O. Messing:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patent held by University of California.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.03/M15

Topic: C.17. Drugs of Abuse and Addiction

Support: Samuel C. Johnson Genomics of Addiction Program

Title: Combined use of acamprosate and escitalopram reduces ethanol consumption in chronically stressed mice

Authors: *M. HO[1], D. J. HINTON[1], J. R. AYERS-RINGLER[1], A. OLIVEROS[1], V. M. KARPYAK[2], D.-S. CHOI[1];
[1]Mol. Pharmacol. and Exptl. Therapeut., [2]Pyshicatry, Mayo Clin., Rochester, MN

Abstract: Depression is a common psychiatric comorbidity of alcoholism which poses major risk to relapse even under treatment. Acamprosate and escitalopram are FDA-approved antidipsotropic agent and antidepressant respectively, but their combined effect on drinking and depression symptoms had not been thoroughly studied. This study aims at investigating whether the combined use of these medications will reduce ethanol consumption and improve depressive-like symptoms in chronically stressed mice. 8-week-old C56BL/6 mice (n = 20) were individually housed and subjected to chronic unpredictable stress paradigm. The stress paradigm consisted of daily exposure to one of the three stresses (forced swim, social defeat and restrain stress) in random order and time. Another group of mice (n = 20; non-stress controls) remained group-housed and were subjected only to normal handling. After 3 weeks of stress, stressed mice demonstrated significantly longer immobility time in forced swim test compared to non-stressed mice (t test $p < 0.05$), indicating a higher degree of hopelessness. All mice were trained for a week to consume 15% ethanol in a two-bottle choice drinking (tap water vs ethanol in tap water) in dark with 2-hour limited access starting from the third hour into the dark phase. In the following week, each stress group were further divided into 4 drug subgroups (n = 5): saline, 200mg/kg acamprosate, 5mg/kg escitalopram and the combination of both were administrated i.p. twice daily (12 hours apart), with one of the injection just preceded the two-bottle choice drinking test under the same setting as training. In stressed mice, only the subgroup administered with the combination of acamprosate and escitalopram showed significantly lower ethanol consumption compared to control; while in non-stressed mice, all drug groups showed significantly lower ethanol consumption (one-way ANOVA $p < 0.05$). At the end of the drug administration week, forced swim test was performed. Stressed mice continued to display longer immobility time than non-stressed mice, but no significant difference was observed among drug subgroups. These results suggest that while both sole and combined administration of acamprosate and escitalopram can reduce binge ethanol consumption in non-stressed mice, only the combined administration is effective in chronically stressed mice, indicating that targeting both glutamatergic and serotoninergic systems simultaneously may be needed for suppressing alcohol consumption in depressed alcoholic individuals.

Disclosures: **M. Ho:** None. **D.J. Hinton:** None. **J.R. Ayers-Ringler:** None. **A. Oliveros:** None. **V.M. Karpyak:** None. **D. Choi:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.04/M16

Topic: B.05. Transporters

Support: NIAAA Grant R01AA019458 (Y.S.)

Title: Ceftriaxone attenuates ethanol drinking and restores extracellular glutamate level through normalization of GLT-1 in nucleus accumbens of male alcohol-preferring rats

Authors: *S. DAS;
Medicinal and Biol. Chem., The Univ. of Toledo, Toledo, OH

Abstract: Alteration of glutamatergic-neurotransmission is a hallmark of alcohol dependence. We have previously reported that chronic ethanol-drinking downregulated glutamate transporter 1 (GLT-1) in nucleus accumbens (NAc) in male P rats in a manner that was reversed by ceftriaxone treatment. However, the effect of ceftriaxone on extracellular glutamate concentrations in NAc after chronic ethanol-drinking has not yet been studied. In the present study, male P rats were treated with ceftriaxone (100 mg/kg/day, i.p.) for five consecutive days following five-weeks of free choice ethanol (15% and 30%) drinking. *In vivo* microdialysis was performed to measure the extracellular glutamate concentrations in NAc and the effect of blockade of GLT-1 with dihydrokainic acid (DHK) on extracellular glutamate in NAc of ceftriaxone-treated rats was determined. Ceftriaxone treatment attenuated ethanol intake as well as ethanol preference. Extracellular glutamate was significantly higher in NAc after five-weeks of ethanol drinking in saline-treated compared to water control rats. Ceftriaxone treatment blocked the increase extracellular glutamate produced by ethanol intake. Blockade of GLT-1 by DHK reversed the effects of ceftriaxone on glutamate and implicated the role of GLT-1 in the normalization of extracellular glutamate by ceftriaxone. In addition, GLT-1 protein was decreased in ethanol exposed animals and ceftriaxone treatment reversed this deficit. Ceftriaxone treatment also increased glutamine synthetase activity in NAc as compared to ethanol drinking saline-treated rats. Our present study demonstrates that ceftriaxone treatment prevents ethanol drinking in part through normalization of extracellular glutamate concentrations in NAc of male P rats via GLT-1. **Keywords:** Alcohol abuse; GLT-1; no-net-flux microdialysis; ceftriaxone; dihydrokainic acid.

Disclosures: S. Das: None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.05/M17

Topic: C.17. Drugs of Abuse and Addiction

Support: IGA NT/14484

PRVOUK P34

Title: Effect of acute injection of methylphenidate (Ritalin) and its combination with morphine on behavior of male rats prenatally exposed to methamphetamine

Authors: *K. NOHEJLOVA, A. YAMAMOTOVA, R. SLAMBEROVA;
Charles Univ. in Prague/ Third Fac. of Med., Prague, Czech Republic

Abstract: Psychostimulant methylphenidate hydrochloride (Ritalin) is used for treatment of attention deficit hyperactivity disorder. It has a chemical structure closely related to the structure of methamphetamine. In the brain it blocks the reuptake of the catecholamines, including dopamine, via binding to its transporter. As a result, amount of available dopamine increases. Dopamine thereafter modulates activity of neuronal system in prefrontal cortex, affecting regulation of cognitive performance and also motor function. The first aim of our study was to compare effect of two doses of Ritalin (1 and 5 mg/kg) on spontaneous behavior in adult male rats prenatally exposed to methamphetamine (MA). The second aim was to analyze further possible changes in behavior when Ritalin is applied in combination with same doses of morphine (MOR). Spontaneous behavior was analyzed using LABORAS system (Metris, The Netherlands). Following parameters were evaluated: locomotion, rearing, average speed of movement and distance moved. Adult male Wistar rats prenatally exposes either to MA or, as control, to saline (Sa) were used in experiment. In the first set of experiments animals received Ritalin in the dose of 1 and 5 mg/kg prior to introduction to LABORAS, where they spent one hour. In the second set, animals received Ritalin with same dose of morphine (Ritalin + MOR at the doses 1 mg/kg; and Ritalin + MOR at the doses 5 mg/kg). Results: (1) higher dose of Ritalin (5 mg/kg) increased animals performance in all of the followed parameters, except of distance moved, regardless prenatal treatment. Bothe doses of Ritalin had more prominent effect in prenatally Sa exposed animals compared to MA exposed ones. Animals prenatally exposed to MA, which received low dose of Ritalin (1 mg/kg) had lowest activity expressed by all parameters. (2) Animals prenatally exposed to Sa, which received higher dose of Ritalin and MOR (5 mg/kg) combination had the highest activity expressed by increase in all parameters. Contrary, animals that were treated with higher doses of Ritalin and MOR, which were prenatally exposed to MA, had the lowest activity. Summarizing, the study suggests that low doses of Ritalin decrease activity in subjects with prenatal to another psychostimulant, while higher dose have opposite effect regardless of prenatal exposure. In addition, it seems that prenatal exposure to Sa in control group had sensitizing effect to psychostimulant, while prenatal exposure to MA had sensitizing effect to morphine. Financial support: #IGA NT/14484 and #PRVOUK P34

Disclosures: K. Nohejlova: None. **A. Yamamotova:** None. **R. Slamberova:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.06/M18

Topic: C.17. Drugs of Abuse and Addiction

Support: IGA NT/14484

Prvouk P34

Title: Acute treatment with methylphenidate (Ritalin) modulates morphine antinociception in rats depending on the dose and tested body site

Authors: *A. YAMAMOTOVA, K. NOHEJLOVA, R. SLAMBEROVA;
Charles Univ, 3rd Fac Med., Prague, Czech Republic

Abstract: Methylphenidate hydrochloride (MPH) is a stimulant drug used for attention deficit hyperactivity disorder treatment and less frequently for management of cancer-related fatigue and sedation. MPH blocks the reuptake of the catecholamines dopamine and noradrenalin by binding to the transporters, thereby increasing catecholamine availability. Noradrenalin acting on noradrenergic receptors can either facilitate or inhibit pain depending on the supraspinal site of release and the type of adrenoceptor activated. The aim of our study was to compare antinociceptive effect of MPH and its combination with morphine (MOR). Antinociceptive effects of MPH and MOR in adult male Wistar rats were investigated by means of withdrawal latency in the plantar test (Ugo Basile, Italy) after single administration of MPH (1 mg/kg or 5 mg/kg s.c.), MOR (1 mg/kg or 5 mg/kg s.c) or their combination. Latencies of withdrawal reflexes of hind limbs and the tail were repeatedly measured before injection of drugs and then three additional times with 15-min intertrial intervals. The percent of the maximal analgesic response defined as (test latency−baseline latency)/(cut-off time−baseline latency)×100% was calculated for each rat on both nociception tasks. On the tail, both doses of MPH were without any antinociceptive effect, whereas 5 mg/kg MPH was effective on the hind limbs. Lower dose of MPH+MOR (1 mg/kg) enhanced antinociception both in the plantar and the tail flick tests in comparison with single doses of MPH or MOR. On the other hand, higher dose of MPH+MOR (5 mg/kg) increased antinociception in the plantar test in comparison with MOR and decreased antinociception in the tail flick test in comparison with MOR. Our research supports the evidence that MPH in lower doses has the ability to enhance the analgesic properties of morphine when both types of drugs are used in combination; however in higher doses it can weaken antinociceptive potency of morphine especially at the spinal site.

Disclosures: A. Yamamotova: None. **K. Nohejlova:** None. **R. Slamberova:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.07/M19

Topic: C.17. Drugs of Abuse and Addiction

Support: GACR 14-03708S

PRVOUK P34

Title: Morphine decreases social interaction of adult male rats, while THC does not affect it

Authors: *R. SLAMBEROVA[1], A. MIKULECKA[2], E. MACUCHOVA[1], I. HREBICKOVA[1], M. SEVCIKOVA[1], K. NOHEJLOVA[1], M. POMETLOVA[1];
[1]Charles Univ., Third Fac. Med., Prague, Czech Republic; [2]Dept. of Developmental Epileptology, Acad. of Sci. of the Czech Republic, Inst. of Physiol., Prague, Czech Republic

Abstract: Psychotropic drugs of abuse are known to have serious influence on humans' as well as animals' behavior. Both, opioids and cannabinoids were shown to affect social behavior in rodents. The aim of the present study was to compare effect of three low doses of morphine (MOR) and delta9-tetrahydrocannabinol (THC) on behavior tested by social interaction test (SIT) in adult male rats. Wistar rats were tested in low stress variant of SIT. 45 minutes prior to testing experimental animals received one of the drugs and doses: MOR (1; 2.5; 5 mg/kg) or THC (0.5; 1; 2 mg/kg). Control rats were injected with solvent (saline for MOR and ethanol for THC). Occurrence and time spent in specific patterns of social interactions (SI) and non-social activities (locomotion and rearing) was video-recorded for 5 minutes and then analyzed by ODLog software (Macropod Software). Morphine in doses of 1 and 2.5 mg/kg displayed decreased SI in total. Detailed analysis of specific patterns of SI revealed decrease in sniffing and allo-grooming after all doses of MOR, and the highest dose (5 mg/kg) of MOR decreased following and increased genital investigation. Climbing was not affected by MOR at all. Regarding to non-social activities: only lower doses of MOR (1 and 2.5 mg/kg) increased rearing activity, although walking was not affected by any of the doses of MOR. THC, in either of the tested doses, did not induce any specific changes in SI or non-social activities, which could be revealed by detailed analysis of behavior, when compared to matching control group (ethanol). However, post-analysis of behavior of the animals showed differences between all THC groups and their ethanol control group when compared to saline controls. It was lower SI in total, lower rearing, but higher sniffing and allo-grooming in THC and ethanol groups than in saline control group. Thus, it seems that this effect is more due to the ethanol than the THC per se. Based on the present results we can assume that opioids affect SI more than cannabinoid. The low tested doses of cannabinoid did not affect evaluated parameters from SIT. This outcome could be the result of ethanol masking an effect of THC.

Disclosures: **R. Slamberova:** None. **A. Mikulecka:** None. **E. Macuchova:** None. **I. Hrebickova:** None. **M. Sevcikova:** None. **K. Nohejlova:** None. **M. Pometlova:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.08/M20

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA036385

Pennsylvania Department of Health Tobacco CURE Funds

Department of Anesthesiology RAP Grant

Title: Sex differences in morphine tolerance and reward in "humanized" A118G mice

Authors: *A. HENDERSON REDMOND[1], M. B. YUILL[2], T. E. LOWE[1], M. L. ZEE[1], J. GUINDON[3], D. J. MORGAN[2];
[1]Dept. of Anesthesiol., [2]Departments of Anesthesiol. and Pharmacol., Pennsylvania State Univ. Col. of Med., Hershey, PA; [3]Dept. of Pharmacol. and Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: The rewarding and analgesic effects of opiates are mediated primarily by the mu-opioid receptor, of which the A118G single-nucleotide polymorphism (SNP) has the greatest link to addiction potential. Clinical and preclinical studies find the G allele associated with increases in heroin reward and self-administration. Clinically, heterozygous individuals expressing one copy of 118G allele report greater pain and reduced responsiveness to opioid drugs after surgery. Taken together, these results suggest that the G allele may confer a genetic vulnerability to opiate addiction and reward. Given the rise in the use of prescription opiates, it is important to understand how the A118G SNP may alter reward, sensitivity, and tolerance to opiates to better treat patients and to also identify those vulnerable to opiate abuse. The purpose of this study was to assess whether the A118G SNP differentially mediates responsiveness to morphine and whether these responses vary as a function of sex. Male and female mice homozygous for the "humanized" 118AA or 118GG alleles were used to test the hypothesis that 118GG mutant mice are less sensitive to the acute and rewarding effects of morphine and developed tolerance to its antinociceptive effects at a faster rate than 118AA wild-type mice. The rewarding effects of morphine were assessed using a conditioned place preference test (CPP). Sensitivity and chronic tolerance to the antinociceptive effects of morphine were examined using the tail-flick, hotplate, and formalin tests. We find that 118AA (but not 118GG) females developed CPP to morphine. While morphine tolerance was not different between 118AA and 118GG mice there was a main effect of sex driven by differences in initial sensitivity to morphine. Interestingly, a challenge dose of morphine two weeks post testing found the effects of tolerance continued to persist in female, but not in male mice. In addition, many of the sex effects observed were driven by differences between the 118AA males and 118GG females, suggesting a genotype interaction

related to sex differences. These results provide further evidence of gender-specific differences between males and females in regards to their sensitivity and tolerance responses to morphine with females exhibiting lower sensitivity to the antinociceptive effects of morphine. In particular, the 118GG females seem to show the least responsiveness to both the antinociceptive and rewarding effects of morphine. Taken together, these data suggest that female 118GG mice may need more morphine to achieve the same degree of antinociception, which could lead to increased susceptibility to opiate abuse.

Disclosures: **A. Henderson Redmond:** None. **M.B. Yuill:** None. **T.E. Lowe:** None. **M.L. Zee:** None. **J. Guindon:** None. **D.J. Morgan:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.09/M21

Topic: C.17. Drugs of Abuse and Addiction

Support: USPHS Grant DA25267

Title: Differential effects of positive nAChR modulators and AChE inhibitors in rhesus monkeys discriminating nicotine

Authors: *M. J. MOERKE, L. R. MCMAHON;
Pharmacol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Galantamine, an acetylcholinesterase (AChE) inhibitor and positive allosteric modulator of nicotinic acetylcholine receptors (nAChRs) that has been approved for use as a cognitive enhancer in humans, has recently demonstrated potential as a smoking cessation aid in pre-clinical assays. One way galantamine might serve as an effective therapy for smoking cessation would be by producing nicotine-like effects. In the current study, a nonhuman primate model of subjective effects was used to examine the extent to which galantamine shares effects with nicotine; moreover, the relative contribution of AChE inhibition and positive allosteric modulation of nAChR to the effects of galantamine was examined. Galantamine was studied in addition to donepezil, another AChE inhibitor which has been approved for attenuation of the cognitive deficits associated with Alzheimer's disease, and PNU-120596, a positive allosteric modulator of nAChRs that produces cognitive enhancement in monkeys. Rhesus monkeys (n=5) discriminating nicotine (1.78 mg/kg calculated as the base weight) responded under a fixed ratio 5 schedule of stimulus-shock termination. Nicotine, galantamine, and donepezil dose-dependently increased nicotine-lever responding; the percentage of nicotine-lever responses was a mean of 98% at 1.78 mg/kg of nicotine, 98% at 1.78 mg/kg of galantamine, and 89% at 0.56 mg/kg of donepezil. The ED50 values (95% confidence limits) were 0.41 (0.1-1.74) mg/kg for

nicotine, 0.77 (0.46-1.28) mg/kg for galantamine, and 0.20 (0.14-0.29) mg/kg for donepezil. PNU-120596, up to a dose of 10 mg/kg, produced a maximum of 1% nicotine-lever responding. Collectively, these results suggest that AChE inhibition and direct nAChR stimulation result in overlapping subjective effects, whereas positive nAChR modulation does not appear to be sufficient to mimic the subjective effects of nicotine. Supported by USPHS Grant DA25267.

Disclosures: **M.J. Moerke:** None. **L.R. McMahon:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.10/M22

Topic: D.17. Voluntary Movements

Support: European Union Project FP7-604102

Austrian Science Fund FWF # I753-N23

Title: Reward-based network plasticity as Bayesian inference

Authors: ***W. MAASS**, D. KAPPEL, S. HABENSCHUSS, R. LEGENSTEIN;
Graz Univ. of Technol., Graz, Austria

Abstract: Experimental data show that synaptic connections, synaptic efficacy, and tuning curves of neurons in are subject to permanently ongoing more-or-less stochastic changes, even in the absence of overt learning tasks. These data raise the question, how stable reward-based learning is possible. We show that these data can be understood from the perspective of a Bayesian learning theory, which posits that networks learn a posterior distribution of network configurations, rather than a single „optimal“ network configuration. In this theoretical framework, the experimentally observed ongoing changes in network configurations (including synaptic connections and synaptic weights) assume an important functional role: They enable the network to sample over time from a low-dimensional manifold of network configurations that have high probability under a posterior distribution of network states, thereby enabling Bayesian inference of network configurations. This posterior results from a prior that enforces structural rules of cortical networks (such as sparse connectivity, specific connection probabilities between specific types of neurons, heavy-tailed distributions of synaptic weights). The other factor of the posterior is a term that reflects the likelihood of a network configuration to lead to rewards (e.g., in the context of some motor learning task). We present a new mathematical framework (employing stochastic differential equations and Fokker-Plack equations) that creates links between local stochastic learning rules and the probability of network configurations under the posterior distribution. In particular, we show that previously proposed rules for reward-gated STDP can be derived from the more general principles of reward-based Bayesian inference of

network configurations. This new model for reward-based network plasticity is not only consistent with experimental data on ongoing stochastic changes in network configurations (in fact: requires such ongoing stochastic changes), but also offers several functional advantages over previously considered learning frameworks based on convergence of the network configuration to an optimal one: --better generalization capability (since the prior works against overfitting of network configurations to a small set of training examples) --automatic compensation for network disturbances or changes in the environment (e.g., reward distributions) --a more more goal-oriented exploration of new network configurations in reward-based learning.

Disclosures: **W. Maass:** None. **D. Kappel:** None. **S. Habenschuss:** None. **R. Legenstein:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.11/M23

Topic: C.17. Drugs of Abuse and Addiction

Support: NHMRC Senior Research Fellowship to MvdB

Title: Modelling methamphetamine psychosis in mice: role of BDNF and reelin

Authors: *M. VAN DEN BUUSE[1], E. E. MANNING[2];
[1]La Trobe Univ., Bundoora, Melbourne, Australia; [2]Florey Inst. of Neurosci. and Mental Hlth., University of Melbourne, Australia

Abstract: The abuse of methamphetamine (METH a.k.a. ice or crystal meth) has reached near-epidemic proportions worldwide. Abuse is particularly prevalent among young adults. METH abuse is associated with a heavy burden on the health care and criminal justice system. Among regular METH users, the prevalence of psychosis is 11 times higher than in the general population. This psychosis closely resembles paranoid schizophrenia with persecution delusions, auditory and visual hallucinations, and social withdrawal. Late adolescence may be a period of particular developmental vulnerability. However, the brain mechanisms involved in vulnerability to methamphetamine psychosis and their overlap with those involved in schizophrenia, remain unresolved. We developed an animal model of methamphetamine abuse and psychosis where mice are treated chronically during late adolescence/young adulthood. The animals receive escalating doses of methamphetamine when they are 6, 7 and 8 weeks of age and the behavioural effects of this chronic 'binge'-like protocol are assessed in adulthood in a range of tests relevant for psychosis-like behaviour, sensorimotor gating, learning and memory, and social behaviour. Because several studies have shown that levels of brain-derived neurotrophic factor (BDNF) and

reelin are reduced by about 50% in post-mortem brain samples from patients with schizophrenia, we used BDNF- and reelin heterozygous mice. BDNF heterozygous mice showed significantly greater sensitization to METH than wildtype control mice and showed selected deficits in social behaviour and spatial memory (Manning & van den Buuse, in preparation). In contrast, after a METH challenge dose, METH-pretreated reelin heterozygous mice tended to show less hyperactivity than similarly pretreated wildtype controls, suggesting a protective effect of partial reelin depletion although male METH-treated reelin heterozygous mice displayed reduced short-term spatial memory. Anxiety was increased in both METH-treated mice controls and reelin heterozygous mice but no differences were found for prepulse inhibition, a measure of sensorimotor gating. These preliminary results reveal involvement of BDNF and reelin in selected behavioural changes in a mouse model of METH abuse and psychosis.

Disclosures: M. Van den Buuse: None. **E.E. Manning:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.12/M24

Topic: F.02. Animal Cognition and Behavior

Support: Quality Initiative Research Grant (Palm Beach Atlantic University)

Title: Modelling the consequences of recreational use of MDMA or 5-MeO-DIPT in humans using a weekend 'rave' exposures

Authors: *F. LUETZENBERG, E. WATKINS, D. M. COMPTON;
Psychology, Palm Beach Atlantic Univ., West Palm Beach, FL

Abstract: Previous research has supported the position that the hallucinogenic "club drugs" 3,4-methylenedioxymethamphetamine (MDMA) and 5-methoxy-N,N-diisopropyltryptamine hydrochloride (Foxy), albeit to different degrees, remain popular as recreational drugs. Much is known about MDMA including observations that in comparison to female rodents, males appear to be more sensitive to the toxic effects associated with abuse. Conversely, less is known about the possible sex differences associated with the abuse of Foxy, especially when the consequences of its use are examined during the neuropsychological development period of adolescence. In the present study, beginning at 35 days of age male and female rats were given multiple doses of MDMA, Foxy (5 mg/kg), or saline across a 48 hour "weekend" under conditions approximating that of a rave. Behavioral testing occurred in adulthood when the rats were 131 days old and had been drug free for 66 days. Assessments included general activity, stepdown passive avoidance, and a series of Morris water maze spatial and nonspatial memory tasks. Depending on task demands, the performance of MDMA-treated rats was inferior to that of the Foxy-treated rats

and saline controls. The performance of both drug groups was comparable and inferior to that of control rats on a spatial learning set task. Generally, greater impairments were observed in MDMA-treated rats than the Foxy-treated rats. Last, sex differences were observed on some but not all spatial tasks with MDMA-treated males performing significantly worse than similarly treated female rats. The results will be discussed in the context of putative sex-mediated differences in sensitivity to MDMA or Foxy. In addition, the disruptive effects of these drugs to central serotonergic systems during adolescence that may contribute to cognitive deficits and maladaptive behavior will be explored.

Disclosures: F. Luetzenberg: None. **E. Watkins:** None. **D.M. Compton:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.13/M25

Topic: C.17. Drugs of Abuse and Addiction

Support: Ministry of Food and Drug Safety (MFDS) of Korea (14182MFDS979)

Title: Methoxetamine conditioned place preference and self-administration in rats: evidence of its abuse potential

Authors: *C. D. BOTANAS[1], J. DE LA PEÑA[1], I. DELA PENA[1], R. TAMPUS[1], H. KIM[1], Y. LEE[2], J. CHEONG[1];
[1]Pharm., Uimyung Res. Inst. For Neurosci., Seoul, Korea, Republic of; [2]Pharm., Lab. of Medicinal Chem., Seoul, Korea, Republic of

Abstract: Methoxetamine (MXE) is a new ketamine derivative synthetic drug, acting as an N-methyl-D-aspartate receptor antagonist. Recently, there have been many reports regarding its misuse in humans that have led to serious or even fatal consequences. Despite these reports, MXE is not regulated in many countries, which may be partly due to the lack of scientific evidence regarding its abuse potential. In the present study, we aimed to evaluate the abuse potential (rewarding and reinforcing effects) of MXE through the conditioned place preference (CPP) and self-administration (SA) tests in Sprague-Dawley rats. In addition, locomotor activity during the conditioning phase of the CPP was also analyzed. Ketamine was used as a reference drug. MXE (2.5 and 5 mg/kg) induced significant CPP in rats, an effect comparable to that of ketamine (5 mg/kg). Interestingly, MXE did not produce any locomotor alterations whereas ketamine decreased the locomotor activity of the rats. In the SA test, the rats showed modest self-administration of MXE (0.25, 0.5, 1.0 mg/kg/infusion), while ketamine (0.5 mg/kg/infusion) was robustly self-administered. These results demonstrate that MXE, similar to ketamine, has rewarding and reinforcing effects in rats, suggesting that the drug has a potential for human

abuse. Furthermore, the discrepant effects of MXE and ketamine in locomotor activity and self-administration rates propose that the psychopharmacological effects of these drugs may diverge in some aspects. More importantly, this study advocates the careful monitoring and prompt regulation of MXE and its related substances.

Disclosures: **C.D. Botanas:** None. **J. de la Peña:** None. **I. dela Pena:** None. **R. Tampus:** None. **H. Kim:** None. **Y. Lee:** None. **J. Cheong:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.14/M26

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01DA035008

NIH T32DA007234

Title: Opposing effects of group I mGluRs on dendritic spine density in the rat nucleus accumbens

Authors: *K. GROSS, R. L. MEISEL, P. G. MERMELSTEIN;
Univ. of Minnesota, Minneapolis, MN

Abstract: The group I mGluRs, mGluR1a and mGluR5, are involved in the development and expression of drug addiction. Manipulation of group I mGluR activity can attenuate behavioral sensitization to drugs of abuse, drug seeking behaviors in models of self-administration, and conditioned place preference, suggesting that modulation of these receptors is a possible avenue for therapeutic treatment. Group I mGluR activity affects both neuronal structure and function in the nucleus accumbens (NAc), which likely plays a role in their effect on addictive behavior. In particular, mGluR5 activity has been associated with synapse elimination in the NAc; however, the role of mGluR1a in structural plasticity in this region is less clear. We sought to compare the effects of mGluR1a versus mGluR5 activation on structural plasticity in the NAc. Rats were given a systemic injection of an mGluR5 positive allosteric modulator (PAM), CDPPB, an mGluR1 PAM, SYN119, or vehicle and were then sacrificed 24 hours later. Neurons in the NAc were ballistically labeled with DiI, and spine densities were determined. Our data revealed an interesting bidirectional effect on spine density, with activation of mGluR5 by CDPPB resulting in decreased dendritic spine density in both the core and shell of the NAc, but mGluR1a activation by SYN119 increasing dendritic spine density in both of these subregions. While changes in spine density were measured 24 hours after drug administration, changes in actin binding proteins and their regulators were observed on a more rapid timescale. These data suggest that positive modulation of different group I mGluRs results in activation of opposing

signaling pathways in the NAc that lead to bidirectional effects on cytoskeletal regulation and dendritic spine density.

Disclosures: K. Gross: None. **R.L. Meisel:** None. **P.G. Mermelstein:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.15/M27

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA035008

Title: Estradiol facilitation of extended access cocaine self administration in female rats requires activation of mGluR5

Authors: *L. A. MARTINEZ, B. M. PETERSON, P. G. MERMELSTEIN;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Females exhibit enhanced responsiveness to the addictive properties of cocaine and several other drugs of abuse. The underlying neural mechanisms that produce this heightened response are not well understood. We have recently found that estradiol decreases dendritic spine density in the core of the female nucleus accumbens, with subsequent enhancement of behavioral sensitization to cocaine, through activation of the metabotropic glutamate receptor mGluR5. The present experiment sought to determine whether mGluR5 activation is also required for the facilitative effects of estradiol on cocaine self administration. To test this hypothesis, ovariectomized female rats treated with estradiol on a 2 days on, 2 days off schedule were initially trained to self administer sucrose pellets (FR1 schedule). Females were then removed from estradiol treatment, implanted with IV catheters, and trained to self administer cocaine (1.5 mg/kg/inf; FR1 schedule). At the completion of cocaine training, females were again restarted on the 2 days on, 2 days off schedule with either oil or estradiol, but with the additional injection of either the mGluR5 antagonist MPEP or vehicle 30 minutes prior to hormone injections. Extended access to cocaine self administration was for 10 days (1.5 mg/kg/inf; 6 hrs per day, FR1 schedule). Females treated with estradiol had significantly higher average daily infusions of cocaine across the 10 days of extended access compared to oil- treated females. This effect of estradiol was completely blocked by MPEP treatment. Furthermore, MPEP treatment alone had no effect on self administration. These data indicate that mGluR5 signaling may be a critical mechanism linking estradiol to enhanced addictive responses in females.

Disclosures: L.A. Martinez: None. **B.M. Peterson:** None. **P.G. Mermelstein:** None.

Poster

322. Behavioral Pharmacology and Modeling in Addiction

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 322.16/M28

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA035008

NSF Grant No. 00006595

Title: In female rat nucleus accumbens, the endocannabinoid system mediates the effects of estradiol on psychostimulant responses and structural plasticity

Authors: *B. PETERSON[1], L. A. MARTINEZ[1], E. LEISHMAN[2], H. B. BRADSHAW[2], R. L. MEISEL[1], P. G. MERMELSTEIN[1];
[1]Neurosci. Dept, Univ. of Minnesota, Minneapolis, MN; [2]Physiological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Estradiol potentiates psychostimulant responses in females, yet the underlying neurobiological mechanisms remain a mystery. Recently our lab has demonstrated that in female rats, the facilitatory effect of estradiol on cocaine-induced locomotor sensitization required activation the metabotropic glutamate receptor mGluR5, and the cannabinoid receptor CB1R. To search for neurobiological underpinnings to these behavioral effects of estradiol, we investigated structural plasticity within the nucleus accumbens (NAc) core. Similar to the observations regarding locomotor sensitization, pretreatment with the mGluR5 antagonist, MPEP (1mg/kg), blocked estradiol-mediated decreases in nucleus accumbens (NAc) core dendritic spine density. In addition, we demonstrate that the estradiol-mediated decrease in dendritic spine density is attenuated by pretreatment with the CB1R inverse agonist, AM251 (1mg/kg). Collectively our data suggest that the effects of estradiol on psychostimulant behavioral responses and NAc core structure require activation of both mGluR5 and CB1R. These data support our model wherein estradiol binds membrane-localized ERα to transactivate mGluR5 in the female rat striatum, leading to CB1R activation. Experiments are currently underway to definitively determine whether estradiol activation of mGluR5 directly mobilizes endogenous cannabinoids (endoCBs) within the NAc.

Disclosures: B. Peterson: None. **L.A. Martinez:** None. **E. Leishman:** None. **H.B. Bradshaw:** None. **R.L. Meisel:** None. **P.G. Mermelstein:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.01/M29

Topic: C.18. Behavioral Pharmacology

Support: NIH Grant DA025679

NIH Grant DA038114

Title: VTA kappa opioid receptor modulation of aversion-induced reductions in dopamine and punishment

Authors: *M. A. ROBBLE, D. S. WHEELER, R. A. WHEELER;
Marquette Univ., Milwaukee, WI

Abstract: Adaptive responses to rewarding and aversive environmental events are critical to survival. Rewarding stimuli and their predictors reliably increase nucleus accumbens (NAc) dopamine signaling which promotes reward based learning. However, less is understood about the involvement of NAc dopamine in aversion learning, and the neural systems that modulate it. Studies monitoring subsecond dopamine release in the NAc have shown that aversive stimuli and their predictors decrease dopamine signaling, and it has been proposed that such reductions are a critical modulator of action selection that promotes avoidance learning. Kappa opioid receptors are expressed on the cell bodies of midbrain dopamine neurons in the ventral tegmental area (VTA), and their activation mediates the anhedonic and dysphoric component of drug withdrawal and other aversion states. Correspondingly, kappa opioid receptor activation has been shown to reduce electrically evoked dopamine release in a NAc slice preparation. Here we examined the neurochemical and behavioral relevance of VTA kappa opioid receptor activation by an aversive stimulus. First, we used fast scan cyclic voltammetry in awake and behaving rats to monitor terminal dopamine release in the nucleus accumbens shell. Following recovery from surgery, rats received intra-VTA infusions of Nor-binaltorphimine (Nor-BNI), a kappa opioid receptor antagonist, or vehicle 24 hours prior to a recording session. During the recording session, carbon fiber microelectrode position in the NAc shell was determined by the observation of spontaneous dopamine transients. Once a suitable recording environment was found, 20 consecutive one minute recordings were taken and served as a baseline. Rats then received 30 intraoral infusions of quinine (0.2ml/infusion, 1inf/min), an aversive tastant, over a 30 minute period. All quinine infusions were recorded with a video camera for subsequent taste reactivity scoring. Passive intraoral infusions of quinine reliably decreased nucleus accumbens dopamine release, and this effect was attenuated by bilateral intra-VTA administration of Nor-BNI. Further analyses of taste reactivity will determine whether Nor-BNI alters the hedonic value of quinine. These results indicate that aversive stimuli decrease nucleus accumbens dopamine signaling, in part, through activation of VTA kappa opioid receptors. Current studies are underway to examine whether VTA kappa opioid receptors mediate the ability of quinine to serve as a punisher in an operant sucrose seeking task.

Disclosures: **M.A. Robble:** None. **D.S. Wheeler:** None. **R.A. Wheeler:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.02/M30

Topic: C.18. Behavioral Pharmacology

Support: NIH Grant MH093650

NIH Grant MH091945

NIH Grant DA030425

T32 Training Grant in Biomolecular Pharmacology GM008541-17

Title: Extended Amygdala PACAP in the behavioral stress response

Authors: ***M. SEIGLIE**, A. IEMOLO, P. COTTONE, V. SABINO;
Dept. of Pharmacol. and Psychiatry, Boston Univ. Sch. of Med., Boston, MA

Abstract: Anxiety-related disorders are the most common forms of mental disorders; characterized by feelings of excessive worry in the absence of specific external stimuli, they are accompanied by physical, affective and behavioral symptoms. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 38-amino acid peptide expressed not only in the hypothalamus but also in various extra-hypothalamic regions including the extended amygdala. PACAP and PAC1 receptor (PAC1R) have been proposed to play a key role in mediating the behavioral and endocrine responses to stress; however, few studies have examined the involvement of the extrahypothalamic PACAP system in the modulation of stress. Our aim was to elucidate the role of the PACAP/PAC1R system of the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) in the context of anxiety-like behaviors. The effects of PACAP microinfusion into either the CeA or the BNST of male rats was assessed in the acoustic startle response (ASR), a behavioral test sensitive to states of anxiety and fear. To test the functional relevance of the endogenous system, the effects of intra-CeA and intra-BNST PACAP antagonist PACAP(6-38) on ASR were assessed, as well as on footshock-induced sensitization of ASR. Infusion of PACAP into both the CeA and BNST increased ASR. Importantly, doses of the antagonist PACAP(6-38) which per se did not affect ASR, were able to prevent the sensitization of ASR by footshock, when infused into both the CeA and BNST. The effects of acute stressors on the expression of PACAP and PAC1R in these brain areas is currently being investigated using immunohistochemistry and real-time PCR. These data prove an anxiogenic role for the PACAP/PAC1 system of the extended amygdala and suggest that hyperactivity of this system may underlie the anxiogenic effects of stress.

Disclosures: **M. Seiglie:** None. **A. Iemolo:** None. **P. Cottone:** None. **V. Sabino:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.03/M31

Topic: C.18. Behavioral Pharmacology

Support: Grant-in-Aid for Young Scientists (B) from Japan Society for the Promotion of Science

Japan Science and Technology Agency for Tokai University

Title: Enhanced motor learning by injecting corticotrophin releasing factor to the cerebellum

Authors: *E. TAKEUCHI[1], M. HIRAISHI[2], A. KATOH[1];
[1]Inst. of Innovative Science, Tokai Univ., Hiratsuka, Kanagawa, Japan; [2]Applied Biochemistry, Sch. of Engin., Tokai Univ., Kanagawa, Japan

Abstract: Corticotropin releasing factor (CRF) is a 41-amino acid peptide. It is secreted from the hypothalamic paraventricular nuclei and regulates the release of adrenocorticotropic hormone. CRF is known to play crucial roles in stress related responses through the hypothalamic-pituitary-adrenal axis and recent studies reported that CRF acts as a neurotransmitter throughout the central nervous system as well. For example, CRF is essential for the induction of long-term depression (LTD) at the parallel fiber - Purkinje cell synapses in the cerebellum. Since the LTD has been thought to be one of the fundamental mechanisms for motor learning, CRF may contribute to motor learning. However, the role of CRF on motor learning *in vivo* still remains unclear. Here, we conducted behavioral studies to examine the effects of CRF on motor learning. We performed rotarod test using Wister rats. The animals injected CRF in the cerebellar cortex were able to stay longer duration on the rotating rod compared to animals injected phosphate buffered saline. The effect was more profound when the rotating speed became faster from 12 rpm to 20 rpm. Interestingly, such enhancement of motor coordination was mainly observed during the early trials. Our results suggest that CRF has a role to acquire the motor learning *in vivo*, especially during the early phase of training.

Disclosures: **E. Takeuchi:** None. **M. Hiraishi:** None. **A. Katoh:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.04/M32

Topic: C.18. Behavioral Pharmacology

Support: NIH Grant K01 DA030444

Title: Dose-dependent reduction of palatable food consumption in binge prone versus binge resistant rats after orexin receptor-1 antagonism

Authors: *K. A. RICHARDSON[1,2], S. UTHAYATHAS[1];
[1]Dept of Pharmacol., Howard Univ. Col. of Med., Washington, DC; [2]Behavioral Neurosci. Branch, Neurobio. of Relapse Section, Natl. Inst. on Drug Abuse, IRP, Baltimore, MD

Abstract: Orexin (or hypocretin) hypothalamic peptides are involved in the regulation of food intake. The purpose of the study was to determine whether there were dose-dependent differences in the consumption of palatable food (PF, high fat, high sugar pellets) in binge-eating prone (BEP) and binge-eating resistant (BER) rats after SB-334867 administration (SB, orexin-1 receptor antagonist). Female Sprague-Dawley rats (freely cycling, 200-300g, n= 6/group) were individually housed and underwent intermittent feeding tests to identify BEP and BER phenotypes. Vaginal lavages were taken for each rat every day and smears were stained to determine the estrous cycle. The BEP and BER phenotypes were based on the consumption of PF pellets. BER rats were those that consistently (> 50% of the time) consumed within the bottom tertile of PF across a minimum of 6 testing days. BEP rats were those that consistently (> 50% of the time) consumed within the top tertile of PF across a minimum of 6 testing days. After phenotypes were identified, animals received either vehicle, 5, 10 or 20mg/kg SB (i.p.). Preliminary analyses show that all three doses of SB reduce PF consumption in BEP and BER rats versus vehicle ($p<0.05$). Dose-dependent reduction in PF consumption is significant in BEP rats and not BER rats when comparing 5mg/kg versus 20mg/kg SB ($p<0.05$). Studies are underway to determine if there are estrous cycle-dependent differences in PF consumption for BEP and BER rats. These data show that antagonism of the orexin system is more effective in reducing consumption of PF in animals that demonstrate binge eating prone behavior.

Disclosures: K.A. Richardson: None. **S. Uthayathas:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.05/M33

Topic: C.18. Behavioral Pharmacology

Support: MH097860 (to WC)

Title: Pituitary adenylate cyclase-activating polypeptide (PACAP) dysregulates social interaction behavior in rats

Authors: *R. J. DONAHUE, A. VENKATARAMAN, E. G. MELONI, W. A. CARLEZON, Jr.;
Dept. of Psychiatry, Harvard Med. School, McLean Hosp., Belmont, MA

Abstract: Severe or prolonged stress can trigger psychiatric illnesses including mood and anxiety disorders. Social withdrawal, defined as diminished interest or participation in social activities, is a core feature of these disorders. Preclinical studies have shown that exposure to a stressful or aversive stimulus (e.g., exposure to predator odor) can disrupt normal social interaction (SI) behaviors in rats, but the mechanisms of this effect are unknown. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a highly conserved neuropeptide that has recently been identified as an important regulator of stress effects and is implicated in the pathophysiology of mood disorders. We recently demonstrated that PACAP administration produces acute increases in anxiety-like behaviors and anhedonia (reduced sensitivity to reward), as well as disruptions in attention and social behavior. In addition, mutant mice lacking the cognate PACAP receptor, PAC1, show reduced aggression and an increase in affiliative behaviors, implicating endogenous PACAP systems in regulating social behavior. Here, we investigated how PACAP (0, 0.25, 0.5, 1.0 μg, administered intracerebroventicularly [ICV]) affects social interaction behaviors. One week after ICV cannula implantation, rats were infused with PACAP and placed in a 60 x 60 x 40 cm Plexiglas chamber with a weight-matched partner rat 1 hr later. Acute PACAP administration produced dose-dependent decreases in active SI (i.e. approach and reciprocal behaviors). Surprisingly, the dose-effect pattern observed at the acute test was reversed when the rats were re-tested without additional treatment 1 week later: rats that had received the highest dose of PACAP (1.0 μg) showed increases in direct SI whereas rats that received the lowest dose of PACAP (0.25 μg) showed nominal decreases in SI and increases in anxiety-like behaviors. This same general pattern (but with more variability) was observed in rats infused with PACAP and tested only at the 1 week time point suggesting that the acute SI exposure is not critical for the development of long-term effects. Preliminary studies suggest that administration of the stress peptide corticotrophin-releasing factor (CRF; 0.5 μg, ICV) also produces acute decreases in active SI, but these effects normalize by the 1 week time point. We are currently examining the role of the transcription factor cAMP response element binding protein (CREB) in mediating the effects of PACAP within the NAc shell and CeA, brain areas involved in encoding reward and aversion. This work may help to devise therapeutics that mitigate specific signs of these disorders by affecting the actions of stress peptides.

Disclosures: R.J. Donahue: None. **A. Venkataraman:** None. **E.G. Meloni:** None. **W.A. Carlezon:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.06/M34

Topic: C.18. Behavioral Pharmacology

Support: Canadian Institute of Health Research to RO

International Brain Research Organization Return Home Grant to JCMM

Title: An neuropeptide Y Y2 receptor antagonist reverses Corticosterone-induced anxiogenic-related behavior and neuronal hypertrophy

Authors: *J. C. MORALES-MEDINA[1,2], I. JUAREZ[5], S. DOMINGUEZ-LOPEZ[3,4], R. KANDIMALLA[2], G. GOBBI[4], G. FLORES[6], R. QUIRION[2];
[1]Ctr. for Res. and Advanced Studies, Tlaxcala, Mexico; [2]Douglas Mental Hlth. Univ. Inst., [3]Dept. of Neurol. & Neurosurg., [4]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada; [5]Facultad de Estomatología, [6]Inst. de Fisiología, Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: Neuropeptide Y (NPY) has been proposed as a potential target for anxiety treatment by modulating its actions through Y1 and Y2 receptors. Indeed, cumulative evidence has shown that NPY modulates corticosterone (CORT) secretion, a key component of anxiety and stress. However, the mechanism of action of NPY on CORT is not completely understood. In particular, acute CORT administration induces an increase in anxiogenic-related behavior in the elevated plus maze (EPM) that parallels with neuronal hypertrophy in the basolateral amygdala (BLA). Whether these CORT effects can be prevented by modulation of NPY receptors has not been studied yet. In the present study, we aimed to investigate the behavioral and neuro-protective effects of the Y1-like receptor agonist, [Leu31Pro34] PYY, the Y1 receptor antagonist, BIBO3304, the Y2 receptor agonist PYY3-36, and the Y2 receptor antagonist, BIIE0246 in CORT-treated rats. While BIIE0246 increased the number of entries in the open arm of the EPM in CORT-treated rats, PYY3-36 decreased the number of entries in control animals. [Leu31Pro34] PYY or BIBO3304 does not produce behavioral modifications in CORT or control rats. Furthermore, BIIE0246 reduced the neuronal hypertrophy in the BLA in CORT-treated rats without inducing neuronal modifications in control rats. Thus, these results showed that BIIE0246 reverses the behavioral and neuronal deleterious effects of CORT treatment and suggest that selective antagonism of the Y2 receptor subtype have a potential use in the treatment of anxiety-related disorders.

Disclosures: J.C. Morales-Medina: None. **I. Juarez:** None. **S. Dominguez-Lopez:** None. **R. Kandimalla:** None. **G. Gobbi:** None. **G. Flores:** None. **R. Quirion:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.07/M35

Topic: C.18. Behavioral Pharmacology

Support: Autism Science Foundation

NIH grant HD071998

Title: Developmental fluoxetine exposure alters affiliative and anxiety-like behavior and oxytocin receptor binding in the prairie vole (Microtus ochrogaster)

Authors: *R. H. LARKE[1,2], M. C. PALUMBO[1,2], S. M. FREEMAN[2], K. L. BALES[1,2];
[1]Psychology, [2]California Natl. Primate Res. Ctr., Univ. of California, Davis, Davis, CA

Abstract: Developmental selective serotonin reuptake inhibitor (SSRI) exposure constitutes a significant risk factor for Autism Spectrum Disorder (ASD). Early manipulation of the serotonin system leads to lasting changes in oxytocin signaling, and the oxytocin system has been linked to ASD. We used the socially monogamous prairie vole as a translational model of developmental SSRI exposure. Twenty female prairie voles were treated with 5mg/kg subcutaneous fluoxetine or saline daily throughout gestation until weaning, so that offspring were exposed to fluoxetine (FLX) or saline (SAL) throughout prenatal and postnatal development. Post-weaning, subjects underwent behavioral testing to detect changes in anxiety-like behavior, sociality, and pair-bond formation; 24 hours later, they were euthanized and brains flash frozen. Quantitative autoradiography was used to measure oxytocin receptor (OTR) density. Preliminary analyses (adult males, N=10) indicate that OTR density in the lateral septum is reduced in FLX animals ($F=5.04$, $p<.05$), and that FLX animals spend more time alone in the partner preference test compared to SAL animals ($F=3.47$, $p<=.05$). FLX increased autogrooming in the open field test ($F=4.46$, $p<.05$), and anxiety-like behavior in the elevated plus maze ($t=2.16$, $p<.05$). Developmental SSRI exposure alters social behavior in the prairie vole in a manner relevant to ASD by increasing anxiety-like behavior and decreasing social interaction. These changes are mediated by alterations to neural OTR density, making the prairie vole a useful translational model for examining serotonin-oxytocin interactions in relation to ASD.

Disclosures: R.H. Larke: None. **M.C. Palumbo:** None. **S.M. Freeman:** None. **K.L. Bales:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.08/M36

Topic: C.18. Behavioral Pharmacology

Support: IRSC Grant 102572

CFI Grant 24326

Title: Rats with antipsychotic-induced dopamine supersensitivity have blunted neurotensin function in the nucleus accumbens

Authors: *A. SERVONNET[1], A.-M. BÉDARD[2], D. LÉVESQUE[3], P.-P. ROMPRÉ[1], A.-N. SAMAHA[2];
[1]Neurosciences, [2]Pharmacol., [3]Pharm., Univ. De Montréal, Montreal, QC, Canada

Abstract: Some of the symptoms of schizophrenia can be treated effectively with antipsychotic drugs. Antipsychotics exert their therapeutic efficacy by occupying striatal dopaminergic (DA) D2 receptors. Although antipsychotics are given to attenuate striatal DA neurotransmission, their chronic use can induce supersensitivity to DA receptor stimulation. This DA supersensitivity can undermine the efficacy of ongoing antipsychotic treatment, alter reward function, and increase the risk of psychosis relapse upon interruption of antipsychotic treatment. The neuropeptide neurotensin is a key dopamine modulator. Within the nucleus accumbens, activation of NTS1 receptors suppresses DA-mediated responses in part by decreasing the agonist binding affinity of D2 receptors. Furthermore, chronic antipsychotic treatment upregulates neurotensin levels in the nucleus accumbens. Here we tested the hypothesis that the expression of antipsychotic-induced DA supersensitivity is linked to altered neurotensin function in the nucleus accumbens. To test this hypothesis, rats were treated with haloperidol for 2 weeks, using a regimen that is clinically representative and that induces sensitization to the behavioural effects of DA agonists (i.e., DA supersensitivity). Three to nine days following treatment cessation, we injected neurotensin into the nucleus accumbens core (0 and 10 μg/0.5 μl/hemisphere) and assessed its ability to suppress amphetamine-induced locomotion. Nine days following antipsychotic treatment cessation, we also assessed the levels of proneurotensin mRNA and NTS1 receptors in striatal subregions. Antipsychotic-pretreated rats showed augmented amphetamine-induced locomotion, indicating that they had developed DA supersensitivity. As shown previously, intra-accumbens injections of neurotensin suppressed amphetamine-induced locomotion in control rats. However, this effect was lost in antipsychotic-pretreated rats. Haloperidol pretreatment elevated proneurotensin mRNA in the caudate-putamen and in the core, but not the shell, of the nucleus accumbens. There were no changes in striatal NTS1 density. Thus, the expression of antipsychotic-induced DA supersensitivity is accompanied by blunted neurotensin function in the nucleus accumbens core and increased proneurotensin transcription in this region. The challenge now is to determine whether normalizing striatal neurotensin function attenuates the behavioural symptoms of antipsychotic-induced DA supersensitivity. These results will lead to a better understanding of the mechanisms underlying DA supersensitivity evoked by antipsychotic treatment.

Disclosures: A. Servonnet: None. **A. Bédard:** None. **D. Lévesque:** None. **P. Rompré:** None. **A. Samaha:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.09/M37

Topic: C.18. Behavioral Pharmacology

Support: NIDDK DK09735

Title: Signaling at Orexin 1 receptors in prelimbic cortex is necessary for Conditioned Saccharin Seeking

Authors: *K. ROACH[1], A. M. CASON[2], G. ASTON-JONES[3];
[1]Allegheny Col., Meadville, PA; [2]Neurosci., Med. Univ. of South Carolina, Charleston, SC;
[3]Rutgers Univ., Piscataway, NJ

Abstract: Previous studies in our laboratory have indicated that signaling at the orexin 1 receptor (OxR1) is involved in cue-induced reinstatement of saccharin-seeking (Cason and Aston-Jones, 2013). However, the precise orexin pathway involved in cue-induced saccharin seeking is still underexplored. Here, we examined the role of the OxR1 in cue-induced reinstatement of extinguished saccharin seeking by examining the effects of local microinfusions of the OxR1 antagonist, SB-334867 (SB), or vehicle into two orexin terminal regions prelimbic cortices (PL) and paraventricular nucleus of the thalamus (PVT). These regions have been previously implicated in hedonic feeding and cue-driven feeding. In the current study, rats were trained to self-administer saccharin (paired with a tone+light cue) and then extinguished as described previously (Cason and Aston-Jones, 2013). Reinstatement of extinguished saccharin seeking was prompted by the appearance of the previously paired tone+light cues. Prior to reinstatement testing, rats were given a bilateral microinfusion (0.3ul) of SB (1mM) or vehicle (artificial cerebrospinal fluid) into PL or PVT. Microinfusions of SB into the PL decreased saccharin seeking behavior during the cue-induced reinstatement sessions compared to vehicle microinfusions. SB was less effective when microinfused into the PVT. The PL result was confirmed by comparing Fos analysis of the PL versus infralimbic (IL) cortices. Systemic SB decreased Fos expression in PL, but not IL, cortex, during cue-induced saccharin seeking. Together these findings indicate that signaling at the OxR1 in the pre-limbic cortex is necessary for cue-induced saccharin seeking.

Disclosures: K. Roach: None. **A.M. Cason:** None. **G. Aston-Jones:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.10/M38

Topic: C.18. Behavioral Pharmacology

Support: MH093981

Title: The effect of orexin receptor blockade on open field behaviors are both sex and age dependent

Authors: *S. R. BLUME[1], S. LUZ[1], D. M. EACRET[1], N. SOTUYO[1], R. J. VALENTINO[1,2], S. BHATNAGAR[1,2];
[1]Abramson Res. Ctr., Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Adolescence is a sensitive and critical period in brain development where psychiatric disorders such as anxiety, depression and post-traumatic stress disorder are more likely to emerge following a stressful life event. Between females and males, females are two times more likely to suffer from psychiatric disorders and show different symptomatology compared to males. Patients suffering from these disorders show alterations in orexins (also called hypocretins), an important neuropeptide synthesized in the lateral hypothalamus that regulates arousal, wakefulness and the hypothalamic-pituitary-adrenal axis. Activation of orexin receptors increases arousal and wakefulness whereas orexin receptor antagonists decrease arousal and wakefulness. However, the role of orexins in mediating behaviors related to arousal in males compared to females and in adolescence compared to adulthood is not known. Here, we examine the influence of orexin receptor blockade (SB334867) in open field behavior in adolescent (PD 31-33) and adult (PD 75-77) female and male rats. Animals were injected with either 10mg/Kg SB334867 (orexin receptor antagonist) or vehicle (i.p.) and placed in the open field 30 minutes post-injection. Behavior was analyzed using the automated behavioral analysis software EthoVision. During the 30 minutes in the open field, orexin receptor blockade significantly reduced the total distance traveled in adolescent females compared to control and drug treated adult females. In contrast, orexin receptor blockade in males showed no differences in distance traveled between age and treatment groups. Preliminary experiments show similar prepro-orexin mRNA levels in control adolescent and adult females, indicating that orexin levels do not explain the differences observed in adolescent and adult females. These results suggest the effect of orexin receptor blockade on open field behavior is both age and sex dependent. Our results also suggest that orexin receptor expression and/or function likely have a developmental maturation that is sex-specific. Current studies are underway to examine additional doses of SB334867 and to determine whether there are differences in orexin receptor expression in adolescent and adult females and males.

Disclosures: **S.R. Blume:** None. **S. Luz:** None. **D.M. Eacret:** None. **N. Sotuyo:** None. **R.J. Valentino:** None. **S. Bhatnagar:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.11/M39

Topic: C.18. Behavioral Pharmacology

Support: FONDECYT Grants Nº 11140065 (GMR)

Committee for Aid and Education in Neurochemistry (CAEN) –International Society for Neurochemistry (ISN)

Title: Reduction of vasopressinergic system following amphetamine sensitization in male and female rats

Authors: *G. M. RENARD, C. AHUMADA, C. BAHAMONDES, R. A. SILVA, G. CRUZ, R. SOTOMAYOR-ZÁRATE;
Univ. de Valparaíso, Valparaiso, Chile

Abstract: Sex differences in the reward circuit response to drugs of abuse are a trending topic in the neurobiology of addiction research. The role of vasopressin (AVP) projections from medial amygdala (MeA) to lateral septum (LS) is well known in different behaviors like anxiety and social behavior that could be altered by drugs. The purpose of this study was to investigate whether amphetamine (AMPH) sensitization affects MeA-LS vasopressinergic system in male and female rats. Male and female Sprague Dawley rats (55-60 days old) were divided in 2 groups: Control rats (0.9% saline solution ml/Kg i.p.) and AMPH-treated rats (AMPH 1.5 mg/Kg i.p.). The stage of the oestrus cycle was determined daily by vaginal smears examination. At the induction phase of sensitization, animals received a daily injection during 5 consecutive days and locomotor activity was measured. The criterion for sensitization was a 20% increase in locomotor activity over 5-day injection period. Five days after the last injection, all rats were injected with AMPH and locomotor activity was measured. AVP mRNA expression in MeA were studied by RT-qPCR and LS AVP levels were also studied by ELISA. Our results showed that approximately 80% of males and females were sensitized. Animals that did not show sensitization to AMPH showed higher locomotor activity at first AMPH injection than sensitized animals. Female rats were sensitized independent of the stage of the oestrus cycle. At the challenge day, the locomotor activity in control rats was higher in females than males. Regarding vasopressinergic system, sensitized rats showed lower levels of AVP mRNA expression in MeA and lower SL AVP than control rats. Thus, our results suggest that AMPH sensitization could alter anxiety and social behavior by reducing the activity of the extrahypothalamic vasopressinergic system.

Disclosures: G.M. Renard: None. **C. Ahumada:** None. **C. Bahamondes:** None. **R.A. Silva:** None. **G. Cruz:** None. **R. Sotomayor-Zárate:** None.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.12/M40

Topic: C.18. Behavioral Pharmacology

Title: Peripheral vasopressin type 1a receptors mediate locomotor inhibition following systemic oxytocin administration in rats

Authors: M. WOLFE, H. WISNIEWSKI, G. IBANEZ, H. TARIGA, D. HARGROVE, *B. F. LINDSTROM;
Ferring Res. Inst., San Diego, CA

Abstract: The neuropeptide oxytocin (OT) has been implicated in a wide range of behaviors in rodents, including pro-social behaviors, analgesia/antinociception, erections, and grooming. OT is not selective for the OT receptor and is known to act at vasopressin type 1a (V1a) and type 2 (V2) receptors. The goals of this study were to (i) evaluate behavioral effects induced by OT in an open-field locomotor assay and (ii) identify receptor subtypes (OT or V1a) mediating these behavioral effects in rats. Behaviors were recorded and analyzed by an automated open-field system designed to sense motion using a grid of perpendicularly placed infrared beams. Both ambulatory (forward movement and rearing) and non-ambulatory (stereotypy) behaviors were measured during 1 hour following administration of OT. Measures of locomotor activity included: distance (cumulative distance traveled by the subject), rearing (number of breaks in the vertical plane), and stereotypic behavior (e.g., scratching, grooming, or digging). Subcutaneous (sc) dosing of OT (0.2 and 1 mg/kg) resulted in a dose-dependent decrease in all locomotor activities measured, however, this effect was absent following intracerebroventricular (icv) administration of OT (30 ng, 5 µl) suggesting that the decreased locomotion was a result of peripheral receptor activation. Interestingly, pre-treatment with the V1a receptor antagonist [Pmp1,Tyr(Me)2]AVP (0.002 and 0.2 mg/kg, sc) dose-dependently blocked the locomotor deficits caused by OT, while pre-treatment with the selective OT receptor antagonist barusiban (0.1 mg/kg, sc) did not. Furthermore, neither barusiban (100 ng, 5µl) nor [Pmp1,Tyr(Me)2]AVP (125 ng, 5µl) given ICV blocked the locomotor inhibition caused by sc administration of OT, further suggesting a peripheral mechanism of action not mediated by the OT receptors, but instead by peripheral V1a receptors. These findings highlight the importance of considering potential off-target effects when using OT in pharmacodynamic studies. We conclude that the exploration of OT pharmacology in the brain and in the periphery is further complicated by the use of non-selective ligands, and that peripheral V1a receptors may underlie many behavioral effects seen following OT dosing.

Disclosures: **M. Wolfe:** A. Employment/Salary (full or part-time):; Ferring Research Institute, Inc. **H. Wisniewski:** A. Employment/Salary (full or part-time):; Ferring Research Institute, Inc. **G. Ibanez:** A. Employment/Salary (full or part-time):; Ferring Research Institute, Inc. **H. Tariga:** A. Employment/Salary (full or part-time):; Ferring Research Institute, Inc. **D.**

Hargrove: A. Employment/Salary (full or part-time):; Ferring Research Institute, Inc. **B.F. Lindstrom:** A. Employment/Salary (full or part-time):; Ferring Research Institute, Inc.

Poster

323. Neuropeptides and Behavior

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 323.13/M41

Topic: C.18. Behavioral Pharmacology

Support: HL112350

HL108475

Title: Defense against social isolation: the study of hormones in an animal model

Authors: T. WISNIEWSKI, N. MCNEAL, W. COLBURN, A. DAGNER, A. TOGHRAEE, M. L. SCOTTI, *A. J. GRIPPO;
Dept of Psychology, Northern Illinois Univ., Dekalb, IL

Abstract: Social stressors, such as social isolation, increase behaviors associated with anxiety and depression and negatively influence physical health in humans. One possible reason for these negative effects might be the result of dysregulation of central oxytocin, which facilitates stress-coping and positive social interactions. To investigate the influence of oxytocin, an animal model that is socially similar to humans is necessary. The prairie vole is a useful model, displaying strong bonds with mates and family members, and exhibiting several negative effects on behavior and health following disruption of social bonds. We investigated the hypothesis that oxytocin antagonism would facilitate depressive and anxiety-like behaviors. 38 female prairie voles were assigned to one of two conditions: socially isolated (separated from a sibling), or a non-isolated, control condition (paired with a sibling) for four weeks. The voles in each group were then treated with either an oxytocin antagonist or saline 1 hour prior to an operational behavioral test. The oxytocin antagonist significantly ($P<.05$) increased anxiety-like behaviors in paired but not isolated animals; whereas it significantly increased depressive behaviors in isolated but not paired animals. These change observed in anxiety behaviors are inconsistent with our previous research. The present research suggests that oxytocin might have varied interactions within the brain as a function of different social environments. Future studies should isolate the specific influence of oxytocin in mediating emotional as well as physiological dysfunction during social stress. Further research involving animal models, such as this, can provide insight into the negative consequences of social stress in humans.

Disclosures: T. Wisniewski: None. **N. McNeal:** None. **W. Colburn:** None. **A. Dagner:** None. **A. Toghraee:** None. **M.L. Scotti:** C. Other Research Support (receipt of drugs, supplies,

equipment or other in-kind support); NIH. **A.J. Grippo:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.01/M42

Topic: C.21.Stroke Recovery

Support: Department of Veterans Affairs

Loyola Neuroscience Division

Title: Investigation of Nogo-A neutralizing antibody treatment and neurogenesis after cortical stroke in adult rats

Authors: D. SHEPHERD[1], S.-Y. TSAI[2], S. P. CAPPUCCI[1], I. VAAGENES[2], V. HUSAK[2], A. MARINOPOULOS[2], R. FARRER[2], *G. KARTJE[2];
[1]Loyola Univ. Chicago, Maywood, IL; [2]Hines VA Hosp, Hines, IL

Abstract: Stroke is a leading cause of adult disability with no approved pharmacological treatments beyond the acute phase. Experimental approaches to improving functional recovery include treatments that enhance plasticity of existing neural circuits or the inherent regenerative capacity of the brain. One such treatment, neutralizing antibodies to the neurite outgrowth inhibitor Nogo-A ("anti-Nogo-A immunotherapy"), has been shown by our laboratory to enhance structural plasticity and functional recovery after stroke in adult and aged rats. However, the exact cellular targets of this novel treatment are not fully understood. A recent study proposed a role for Nogo-A in the maintenance of the subventricular zone (SVZ), one of the adult brain's main neurogenic niches (Rolando et al., J Neurosci, 2012). Endogenous neural precursor cells may contribute to injury mitigation and behavioral recovery after stroke, raising the intriguing question of whether neural precursor cells are a therapeutic target of anti-Nogo-A immunotherapy. To investigate this possibility, we induced cortical ischemia in rats via middle cerebral artery occlusion, followed by anti-Nogo-A antibody treatment delivered by osmotic minipump into the ipsilesional lateral ventricle. At three time points, cellular proliferation in the SVZ was measured by bromodeoxyuridine injection and stereological cell counting, and the neuroblast response was assessed using quantitative immunohistochemistry. We confirmed Nogo-A expression by doublecortin-positive neuroblasts in the SVZ of both sham and ischemic brains. However, we found no evidence of changes in proliferation after either 3, 7, or 14 days of anti-Nogo-A treatment, and no evidence of a treatment effect on neuroblast density. We are currently investigating whether Nogo-A neutralization affects longer-term parameters of post-stroke neurogenesis, including the differentiation, integration, and survival of newborn neurons.

Disclosures: **D. Shepherd:** None. **S. Tsai:** None. **S.P. Cappucci:** None. **I. Vaagenes:** None. **V. Husak:** None. **A. Marinopoulos:** None. **R. Farrer:** None. **G. Kartje:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.02/M43

Topic: C.21.Stroke Recovery

Title: Acute effects of ß-hydroxybutyrate (BHB) in CD-1 mice after experimental stroke

Authors: ***K. A. KOCH**[1], A. THINNES[2], D. BERRESSEM[3], J. BARNSTORF-BRANDES[2], G. ECKERT[3], J. KLEIN[3];
[1]Goethe Univ. Frankfurt Am Main, Frankfurt Am Main, Germany; [2]Pharmacol., [3]Goethe Univ., Frankfurt am Main, Germany

Abstract: (R)-3-Hydroxybutyrate (BHB) is the most important ketone body. In the present study, we have used an experimental stroke model, middle cerebral artery occlusion (MCAO), in mice to investigate the levels of metabolites linked to BHB after brain ischemia. In female CD-1 mice, we used a transient model (90min). Immediately afterwards, mice were injected with 10, 30 or 100 mg/kg BHB. After 22h of reperfusion, mice underwent several motoric and cognitive tests. Two hours later, mice were sacrificed, and tissue were harvested. Sample preparation included extraction of metabolites and derivatisation prior to GC-MS analysis. We found that mice who had received 30 mg/kg BHB performed significantly better than the control group in behavioral tests (neurological score NSS 5.0 ± 1.2 for BHB; 13.1 ± 0,6 for saline) (all data given as means ± S.E., N=11). Both 10 and 100 mg/kg BHB doses gave worse results than the intermediate dose of 30 mg/kg. BHB plasma levels were increased in stroked mice (sham group 181±24µM; stroked group 5.56±1.61mM) but was reduced by 30 mg/kg BHB application (2.31±0.51 mM). With respect to energy metabolites, plasma lactate levels were increased after stroke (sham group 3.41 ±0.34mM; stroked group 9.47±0.73mM) but BHB (30mg/kg) application kept lactate levels at low values (1.98±0.42mM). In contrast, plasma pyruvate levels were decreased in stroked mice (sham group 116.7±17.6µM; stroked group 17.5±3.9µM). Glucose levels were increased 24h after stroke (sham group 10.0±1.4mM; stroked group 20.0±3.8mM) but attenuated by BHB to 13.3±2.7mM. In brain homogenates, BHB levels were 118 24.8 µM in sham-operated mice (calculated with 70% intracellular water). BHB levels were higher in the brains of stroked mice (321±97µM) and even higher after additional BHB administration (459±79µM) Furthermore, 24 h after stroke, lactate levels in brain homogenate were unchanged by stroke (sham group 19.8±1.65mM; stroked group 20.0±4.33mM) but increased after 30 mg/kg BHB (30.0±1.96mM. Finally, glucose levels (43.0±8.3µM in sham-operated mice) were increased after stroke (435±189µM) and further increased after BHB

treatment (771±203 μM). In conclusion, brain ischemia causes an increase of all energy metabolites in blood plasma, with a particularly strong effect on BHB levels. In the brain, stroke causes an increase of glucose and BHB concentrations, two nutrients that may be used alternatively by brain cells. Additional BHB application causes a further rise of BHB in the brain which causes a accumulation of glucose, possibly by a preference of the brain for BHB oxidation. The high lactate levels may be a consequence of brain hypermetabolism 24 h after transient ischemia.

Disclosures: K.A. Koch: None. **A. Thinnes:** None. **D. Berressem:** None. **J. Barnstorf-Brandes:** None. **G. Eckert:** None. **J. Klein:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.03/M44

Topic: C.21.Stroke Recovery

Support: General Grant of NSFC 31371092

Key Project Grant of NSFC 31330035

HKGRF Grant 466713

HMRF Grant 01120146

Title: Mechanisms of dopaminergic drug treatment on motor recovery in ischemic stroke injury

Authors: *L. YAN, L. H. XU, Y. H. LIU, Q. LI, W. H. YUNG, Y. KE;
Sch. of Biomed. Sci., The Chinese Univ. of Hong Kong, Hong Kong, China

Abstract: The development of effective treatment for ischemic stroke is a vexing challenge for centuries, especially for chronic patients where neuroprotective and thrombolytic agents available do not promote functional recovery. Recent clinical studies have shown that administration of dopamine agonists or restoring central dopamine level via levodopa, can substantially improve motor performance in stroke patients. However, the mechanisms of dopaminergic treatment on motor recovery have not been thoroughly explored. In our study, we tested the hypothesis that levodopa acts by enhancing cortical neuroplasticity to promote functional recovery. In rodent model of focal ischemic stroke induced by photothrombosis, we assessed the integrity of cortical dopaminergic system and found that it is significantly disrupted by focal stroke. Through a 3-week daily treatment of levodopa, we recapitulated its beneficial effects on sensorimotor function by subjecting the animals to various motor tasks, including open-field test, rotarod performance test, forelimb food retrieval task, limb-use asymmetry test and horizontal ladder test. Further examination of neuronal connectivity and synaptic protein

expression revealed that perilesional tissue remodeling occurs following stroke and is modulated by dopamine. With tantalizing evidence that dopamine replacement can enhance functional recovery by coalescing connections across the cortex, we demonstrated that the modulating dopaminergic transmission is a promising therapeutic strategy for stroke rehabilitation.

Disclosures: L. Yan: None. **L.H. Xu:** None. **Y.H. Liu:** None. **Q. Li:** None. **W.H. Yung:** None. **Y. Ke:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.04/M45

Topic: C.21.Stroke Recovery

Support: DZHK

Title: Aleglitazar therapy decreases microglia activation and leads to neuroprotective effects after focal brain ischemia in mice

Authors: V. BOUJON, G. KRONENBERG, R. UHLEMANN, M. ENDRES, *U. K. DIRNAGL, K. GERTZ;
Charite Universitätsmedizin Berlin, Berlin, Germany

Abstract: Cerebral ischemia leads to neuroinflammation by triggering multiple inflammatory pathways affecting the entire neurovascular unit. Microglia are a key component of the brain's inflammatory response. In this project, we studied the effects of aleglitazar, a new dual PPARα and PPARγ agonist, on the properties of microglia and on post-stroke outcome. 129/SV mice were subjected to filamentous middle cerebral artery occlusion (MCAo)/reperfusion. Animals were treated with aleglitazar or vehicle once per day, starting from reperfusion. Acute lesion sizes were measured on day 7 after 30 min MCAo. Additionally, the effects of aleglitazar on microglia phenotypes were tested in well established *in vitro* assays. Lipopolysaccharide (LPS)-challenged primary and BV-2 microglia were treated with aleglitazar. Nitric oxide (NO) production was quantified as nitrite accumulation using the Griess reagent for nitrite. Transcription and release of pro-inflammatory cytokines were assessed by quantitative RT-PCR and enzyme-linked immunosorbent assays (ELISAs). Proliferation, migration and phagocytosis of microglia were assessed by Fluorescence Activated Cell Sorting (FACS) analysis, Boyden-Chamber assay and phagocytosis of E. coli bioparticles, respectively. Aleglitazar reduced the mRNA expression of inducible nitric oxide synthase (iNOS), Nuclear Factor kappa B (NfκB) and of tumor necrosis factor (TNF)α, as well as the release of interleukin 6 (IL-6) and TNFα into the culture medium of LPS-challenged microglia. Furthermore, treatment with aleglitazar significantly decreased the proliferative, migratory and phagocytic properties of microglia, as

well as their level of NO secretion. At the concentrations used, aleglitazar did not affect viability of microglia. In addition, aleglitazar efficiently reduced the mRNA transcription of pro-inflammatory marker genes including iNOS and interleukin-1 (IL-1)β after brain ischemia. Moreover, aleglitazar treatment significantly reduced the infarct volume at 7 days after MCAo/reperfusion. Our results provide first evidence that treatment with the new dual PPARα and PPARγ agonist aleglitazar decreases LPS-induced microglia activation, reduces the expression of pro-inflammatory molecules and protects from brain ischemia *in vivo*. Therefore, pharmacological treatment with aleglitazar represents a promising approach to reduce neuroinflammation, thereby affording neuroprotection after ischemic stroke.

Disclosures: V. Boujon: None. **G. Kronenberg:** None. **R. Uhlemann:** None. **M. Endres:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; F. Hoffmann-La Roche. **U.K. Dirnagl:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; F. Hoffmann-La Roche. **K. Gertz:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; F. Hoffmann-La Roche.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.05/M46

Topic: C.21.Stroke Recovery

Support: Grant of Ministry of Health and Welfare B110072

Title: The multi-herbal mixture HT047 promotes motor recovery in a rat model of chronic stroke

Authors: *J. SONG, D. LEE, Y.-S. KIM, S. LEE, H. KIM, H. LEE, H. GUO, H. KIM; Kyunghee Univ., Soeul, Korea, Republic of

Abstract: HT047 is a multi-herbal mixture consisting of Pueraria lobata root and Scutellaria baicalensis root which have been used together to treat stroke in traditional Asian medicine. In the present study, the effects of HT047 on motor recovery in chronic stroke were investigated. Sprague-Dawley rats subjected to 60 min of middle cerebral artery occlusion (MCAO) were randomly divided into two groups, control and HT047 group. Twenty-four hours after MCAO, HT047 was orally administered to rats for 14 days at a dose of 300 mg/kg and then rats were received chow diet containing 1.35 % HT047 up to 6 months. Rats were weighed daily over 6

months after MCAO. Functional recovery was assessed during subacute (weekly intervals for one month) and recovery (monthly intervals up to 6 months) phases using rotarod test and limb placement test. In rotarod test, rats were trained for 7 days prior to MCAO. The latency to fall on the accelerating rotarod was recorded for 5 min. Rotarod performances were expressed as a percentage of the performance of the day before ischemia. In limb placement test, rats were held by the examiner and forelimb placing was noted when approaching a table or table edge from various directions with and without allowing visual, tactile, and proprioceptive support as earlier described by De Ryck et al. (1989). The maximum score was 14 for each body side. After MCAO, the mean weight loss of rats in control group peaked at Day 4, with a maximum loss of 14.8% of the original body weight recorded on the day of surgery. The mean weight of HT047 group was significantly higher than that of control group at 4 days post-surgery (274.3 ± 4.2 vs. 258.0 ± 6.0 g, $p<0.05$). Treatment with HT047 markedly enhanced the recovery on body weight loss after MCAO surgery. In rotarod test, HT047 accelerated recovery of motor function during a subacute period and protected age-related decline during a chronic recovery phase. Rats treated with HT047 showed significant improvement of rotarod performance at 1, 2, 3 and 4 weeks post-surgery compared to control group. At week 4, rotarod performance for each group was 63.2 ± 7.8 % for control group and 88.6 ± 5.7 % for HT047 group. In limb placement test, scores of HT047 group were significantly higher than that of control group during subacute period. At week 4, score for each group was 4.6 ± 0.6 points for control group and 8.7 ± 0.8 points for HT047 group. These results suggests that HT047 treatment prevents weight loss and improves functional recovery after cerebral ischemia.

Disclosures: J. Song: None. **D. Lee:** None. **Y. Kim:** None. **S. Lee:** None. **H. Kim:** None. **H. Lee:** None. **H. Guo:** None. **H. Kim:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.06/M47

Topic: C.21.Stroke Recovery

Support: NSC 99-2320-B-001-016-MY3

NSC 100-2311-B-001-003-MY3

Title: Effectiveness of targeting P2X7 receptor for the treatment of central post-stroke pain in rat model

Authors: *Y.-H. KUAN[1], H.-C. SHIH[1], S.-C. TANG[2], J.-S. JENG[2], B.-C. SHYU[1]; [1]Neurosciences, IBMS, Taipei, Taiwan; [2]Dept. of Neurol. and Stroke Ctr., Natl. Taiwan Univ. Hosp. and Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan

Abstract: Stroke is a leading cause of death and disability in industrialized countries. Approximately 8-14% of stroke survivors suffer from central post-stroke pain (CPSP) when hemorrhagic stroke occurs in lateral thalamic regions, which severely affects their quality of life. Because the mechanisms of CPSP are not well understood, effective treatments have not been developed. In the present study, we tested the hypothesis that persistent CPSP is caused by P2X7 receptor activation after brain tissue damage and subsequent elevations in inflammatory cytokines. A thalamic hemorrhagic rat model was used, characterized by thermal and mechanical allodynia that develops in the subacute to chronic phases upon CPSP onset. We found a significant increase in P2X7 expression in reactive microglia/monocyte-drived macrophages in thalamic peri-lesion tissues at 5 weeks post-hemorrhage. Thalamic P2X7 receptors were directly involved in pain transmission and hypersensitivity. The systemic targeting of P2X7 receptors during the acute stage of hemorrhage rescued abnormal pain behaviors and neuronal activity in the thalamocingulate pathway by reducing reactive microglia/monocyte-drived macrophages aggregation and associated inflammatory cytokines. After CPSP onset, the targeting of interleukin-1β reversed abnormal pain sensitivity. The aberrant spontaneous thalamocortical oscillations in rats with CPSP were modulated by blocking P2X7 receptors. Taken together, our results suggest that targeting P2X7 may be bi-effective in the treatment of CPSP, as both a pain blocker and immunosuppressant that inhibits inflammatory damage to brain tissue. P2X7 receptors may serve as a potential target to prevent the occurrence of CPSP and may be beneficial for the recovery of patients from stroke.

Disclosures: **Y. Kuan:** None. **H. Shih:** None. **S. Tang:** None. **J. Jeng:** None. **B. Shyu:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.07/M48

Topic: C.21.Stroke Recovery

Support: Swedish Medical Center

Craig Hospital

Colorado Neurological Institute

Title: The arginine-vasopressin receptor blocker Conivaptan reduces stroke-evoked brain edema and blood-brain-barrier disruption

Authors: *S. M. JONES[1], E. ZEYNALOV[1], J. SEO[1], L. SNELL[2], J. ELLIOTT[3];
[1]Neurotrauma Res., Swedish Med. Ctr., Englewood, CO; [2]Colorado Neurolog. Inst., Englewood, CO; [3]Colorado Brain and Spine Inst., Englewood, CO

Abstract: Stroke is the fourth leading cause of death and the number one leading cause of disability in the US. Stroke is often associated with the syndrome of inappropriate release of antidiuretic hormone (SIADH). SIADH is the result of uncontrolled arginine-vasopressin (AVP) secretion which causes water retention in the body, hyponatremia, and low plasma osmolality. These events can quickly exacerbate post-ischemic brain edema and increase mortality rate if plasma sodium levels and osmolality are not corrected Conivaptan (Vaprisol) is an antagonist of both AVPR1a and AVPR2 receptors, available for clinical use to correct hyponatremia, blood volume and osmolality. As an AVPR1a receptor blocker, Conivaptan may prevent vasoconstriction and platelet aggregation, effects which can directly benefit regional cerebral blood flow (rCBF) after stroke. Recent literature suggests that selective AVPR1a receptor blockers can reduce post-ischemic brain edema formation, provide neuroprotection, and decrease BBB disruption. The AVPR2 blocking effect of Conivaptan is responsible for excretion of excess water by the kidney and elevation of blood osmolality, which could also alleviate brain edema. Therefore, we conducted a study to investigate whether Conivaptan can prevent brain edema and BBB disruption in mice after stroke. **Methods:** C57/BL6 mice underwent the filament model of middle cerebral artery occlusion (MCAO) with reperfusion. Continuous IV infusion with Conivaptan or normal saline (NS) was initiated immediately at reperfusion and administered via a catheter in the jugular vein for 48 hours. For comparison, the AVPR2 antagonist Tolvaptan was administered p.o. immediately and again 6 hours after reperfusion. Physiological variables including neurological deficit scores, serum and urine osmolality and sodium levels were recorded during the experiment. Brain water content (BWC) and Evans Blue (EB) extravasation index were evaluated at the end point. **Results:** BWC was increased in the ipsilateral hemisphere of vehicle treated mice (81.66 ± 0.43%). Treatment with Conivaptan reduced BWC to 80.84 ± 0.56% (Conivaptan, 0.02 mg), and 78.28 ± 0.48% (Conivaptan, 0.2 mg, $p < 0.05$ vs NS). Conivaptan also reduced the EB extravasation index from 1.22 ± 0.08 (NS) to 1.01 ± 0.02 (Conivaptan, 0.2 mg, $p < 0.05$). **Conclusion:** Continuous IV infusion with Conivaptan for 48 hours after experimental stroke reduces brain edema, and BBB disruption. Conivaptan may potentially be used in patients to alleviate brain edema after stroke.

Disclosures: **S.M. Jones:** None. **E. Zeynalov:** None. **J. Seo:** None. **L. Snell:** None. **J. Elliott:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.08/N1

Topic: C.21.Stroke Recovery

Support: JSPS KAKENHI 15H01445

JSPS KAKENHI 25750213

JSPS KAKENHI 15K16361

Title: Changed profile of glutamate receptors by constraint-induced movement therapy links to functional recovery of the forelimb in capsular hemorrhage rats

Authors: *A. ISHIDA, Y. UEDA, C.-G. JUNG, K. ISHIDA, H. HIDA;
Nagoya City Univ. Grad. Sch. of Med. Sci., Nagoya, Aichi, Japan

Abstract: Constraint-induced movement therapy (CIMT), a method for intensive use of an impaired forelimb, is an effective poststroke rehabilitation to promote functional recovery, although an immediate focused training could induce excitotoxicity deteriorating brain injury after stroke. To investigate the relationship between CIMT and excitatory glutamate receptor profile, male Wistar rats (200-250 g) were given to collagenase (type IV, 15 U/ml, 1.4 μl) to make small hemorrhage near the internal capsule (ICH), and allowed to CIMT for a week with a one-sleeve cast forcing the use of only impaired forelimb in all daily activities. Four groups were prepared: ICH with no CIMT (ICH), ICH with CIMT in early phase (1-8 days post ICH, E-CIMT) or in late phase (17-24 days, L-CIMT), and ICH with forced-nonuse of an impaired forelimb (1-8 days post ICH, E-FNU). It was revealed that protein expressions of NMDA-type receptor subunits, especially NR2A and 2B, were significantly increased in affected motor cortex in E-CIMT compared to ICH group, while those were decreased in the same area in E-FNU group. On the other hand, expressions of AMPA receptors were not changed among groups. More αFosB-positive cells in the motor cortex of ipsi-lesional side indicated enhanced neuronal activity after ICH in E-CIMT group. To investigate the involvement of NMDA receptor signaling in E-CIMT group, NMDA receptor antagonist MK-801 (10 pmol/ml/h, 7 days) was administrated to the motor cortex of ipsi-lesional side using osmotic mini-pump. Although E-CIMT group showed better functional recovery in the reaching and ladder stepping test, the recovery was apparently blocked by MK-801 injection during CIMT. These data suggest that CIMT from early phase after ICH changes the profile of NMDA-type receptor signaling in the motor cortex of ipsi-lesional side, probably affecting the functional recovery by CIMT

Disclosures: **A. Ishida:** None. **Y. Ueda:** None. **C. Jung:** None. **K. Ishida:** None. **H. Hida:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.09/N2

Topic: C.21.Stroke Recovery

Title: Effect of sensorimotor intervention on norepinephrine levels in the hippocampal dentate gyrus and in the pons in adult rats with cortical ablation induced damage

Authors: G. A. GARCÍA-DÍAZ[1], L. E. RAMOS-LANGUREN[2], A. GONZÁLEZ-MACIEL[3], A. BUENO-NAVA[4], A. ÁVILA-LUNA[4], N. CHAVEZ-GARCÍA[5], *S. MONTES[5], R. GONZALEZ-PIÑA[4];
[1]Escuela Superior de Medicina. Maestría en ciencias de la Salud, Inst. Politecnico Nacional. Mexico, Mexico City, Mexico; [2]Sistémicas Biológicos, Univ. Autónoma Metropolitana Unidad Xochimilco, Mexico City, Mexico; [3]Inst. Nacional de Pediatría. Mexico, Mexico City, Mexico; [4]División de Neurociencias, Torre de Investigación, Inst. Nacional de Reabilitación. Mexico, Mexico City, Mexico; [5]Natl. Inst. Neurol. Neurosurg, Mexico City, Mexico

Abstract: Nowadays, a consensus has been reached that designates the functional and structural reorganization of synapses as the primary mechanisms underlying the process of recovery from brain injury. We have reported that pontine norepinephrine (NE) is increased in animals after cortical ablation (CA). Anatomical relationships between the dorsal pontine locus coeruleus (LC) and the dentate gyrus (DG) have been described. To find out whether the increase of pontine NE during recovery is accompanied by changes on NE content in the DG, we measured the NE levels from both structures. Additionally, we analyzed structural modifications in the LC and in the DG in adult rats 20 days after CA. Rats were introduced to 15 sessions of sensorimotor intervention (SMI) as an enhancing recovery procedure. The animals were randomly divided into 4 groups: sham-operated (Sh group) motor cortex injured (Inj group), sham-operated with SMI (Sh+SMI group), and motor cortex injured with SMI (Inj+SMI group). The injured groups underwent cortical damage by means of the aspiration of the tissue of the right motor cortex. In the Inj+SMI group, animals were introduced to SMI one day after CA. All groups underwent histological analysis. Injured rats showed reduction in the number of granule cells in the DG and decreased dentate granule cell layer thickness. Importantly, after SMI, the loss of granule cells was prevented. No differences in the number of cells in the LC were observed among the test groups. On the one hand, NE content in the DG diminished in the Inj group versus controls, on the other hand Inj+SMI group statistically increased NE content as compared to Inj group. Pontine NE levels were not different among groups, suggesting NE pontine recovery. Our results suggest that rehabilitory intervention in the form of sensory motor stimulation elicits structural modifications in the hippocampus that could reorganize the system and lead the recovery process, modulating structural and functional plasticity.

Disclosures: G.A. García-Díaz: None. **L.E. Ramos-Languren:** None. **A. González-Maciel:** None. **A. Bueno-Nava:** None. **A. Ávila-Luna:** None. **N. Chavez-García:** None. **S. Montes:** None. **R. Gonzalez-Piña:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.10/N3

Topic: C.21.Stroke Recovery

Support: Nutricia Research

Title: A specific multi-nutrient intervention as therapeutic approach for stroke

Authors: L. M. BROERSEN[1,2], M. WIESMANN[3,4], B. ZINNHARDT[5], M. HELLWICH[3], S. ELIGEHAUSEN[5], D. REINHARDT[5], A. HEERSCHAP[4], *P. KAMPHUIS[1,2], J. C. CLAASSEN[4], A. J. KILIAAN[3];
[1]Nutricia Res., Utrecht, Netherlands; [2]Utrecht Inst. for Pharmaceut. Sci., Utrecht, Netherlands; [3]RadboudUMC, Nijmegen, Netherlands; [4]Donders Inst. for Neurosci., Nijmegen, Netherlands; [5]European Inst. for Mol. Imaging, Münster, Germany

Abstract: Occlusion of the middle cerebral artery (MCAo) is the most common cause of ischemic stroke in human. Cerebral ischemia leads to brain lesions existing of an irreversibly injured core and ischemic boundary zone, the penumbra, containing damaged but potentially salvageable tissue. Using a transient MCAo mouse model we investigated the effect of a specific multi-nutrient intervention as a therapeutic approach to counteract impairments of cerebral connectivity, cerebral blood flow (CBF), cognition, motor function and neurodegeneration. This specific experimental diet was developed to support neuronal membrane synthesis and maintenance of vascular health. Male C57BL/6j mice (3 months) were subjected to transient (30 min) right MCAo using the intraluminal filament model. Success of surgery was verified intraoperatively. Before tMCAo, baseline measurements of motor parameters (open field, rotarod, grip test, pole test) and spatial learning/memory (Water Maze) were performed and repeated after tMCAo. Starting directly after tMCAo, animals were fed the control diet or the experimental diet. At 14 and 35 days after tMCAo, MRI scanning was conducted to identify stroke location and size (RARE+DWI), and functional and neuronal connectivity and CBF measures (rsfMRI, DTI, MRS, FAIR-ASL) on the 11.7T magnet (Bruker BioSpec). Directly following the MR measurements, brains were collected and immunohistochemical analyses were performed. Data processing is ongoing and final results will be presented. First results show that at 7 days after tMCAo, all mice showed a decreased CBF in the occluded cerebral hemisphere compared to the control hemisphere. Mice fed experimental diet had a higher cortical CBF in the ischemic hemisphere than control fed mice. 35 days post tMCAo, experimental diet fed animals had a restored cortical and hippocampal CBF in the ischemic hemisphere. On control diet, cortical and hippocampal CBF remained decreased in the occluded cerebral hemisphere compared to the control hemisphere. The first functional benefits of the experimental diet were noted in the open field, where locomotor activity was improved at 16 days after tMCAo as compared to animals on the control diet. No therapeutic intervention is available for stroke yet. Our present data show that a specific dietary intervention has beneficial effects on the cerebral hemodynamics after tMCAo. In addition, the diet may reduce some of the functional consequences of tMCAo. Together our data indicate that specific multi-nutrient interventions

may be able to counteract harmful effects of ischemic stroke, protecting against neuronal and connectivity loss and accompanying functional deficits.

Disclosures: **L.M. Broersen:** A. Employment/Salary (full or part-time):; Nutricia Research. **M. Wiesmann:** None. **B. Zinnhardt:** None. **M. Hellwich:** None. **S. Eligehausen:** None. **D. Reinhardt:** None. **A. Heerschap:** None. **P. Kamphuis:** A. Employment/Salary (full or part-time):; Nutricia Research. **J.C. Claassen:** None. **A.J. Kiliaan:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.11/N4

Topic: C.21.Stroke Recovery

Support: PROAPARC/UNASP-SP

Title: Shiatsu improves functional motor capacity in post-stroke patients

Authors: ***H. C. MARCUSSO**[1], S. M. TAGAMI[3], D. PARIZOTTO[2], D. M. DOS SANTOS[2], R. N. ISAYAMA[3];
[2]Human Morphophysiology, [1]UNICASTELO, Fernandopolis, Brazil; [3]Physiotherapy, UNASP-SP, Sao Paulo, Brazil

Abstract: Stroke is an important cerebrovascular disease to cause morbidity and mortality in Brazil and worldwide. Motor function and cognition are mostly impaired in post-stroke patients, resulting in reduced functional capacity that compromises their quality of life. Functional and emotional recovery is variable and depends on the severity of the injury, socio-cultural factors and therapeutic strategies. Shiatsu is an ancient technique of complimentary therapy based on non-invasive points of pressure that are usually at the anatomical sites of muscle-tendon transition or close to motor points where muscles are effectively stimulated by electrodes. Finger pressure using as a shiatsu technique employed on spastic muscles could ameliorate spasticity by stimulating Golgi tendo organs. Such structures, specially regulate muscular tone of rigid muscles. Preview studies suggest that shiatsu mitigates sequel of stroke. Although cerebrovascular injuries cause primary lesion of the upper motor neuron, interventions in peripheral neuromuscular structures may also benefit muscle tone and performance, by increasing the functionality and quality of life of these patients. The aim of this study was evaluating the beneficial effects of shiatsu on functional capacity and quality of life in post-stroke chronic patients. This study was approved by the ethics committee. Six (n=6) post-stroke patients were assigned for shiatsu-kinesiotherapy group (SHI) with pressure points in upper and lower limbs. A control kinesiotherapy group (CRT, n=6) received conventional kinesiotherapy and both groups were treated twice a week for four months. Patients responded to SF-36

questionnaire and Barthel index prior and after all therapeutic procedures. The results showed that shiatsu and/or kinesiotherapy did not change blood pressure, mood, consciousness or caused discomfort in post-stroke patients. SF-36 revealed that SHI group had improved their pain management, vitality, social and the mental health state ($p < 0.05$) comparing to its control. Barthel index showed a recovery in SHI group from severe to moderate dependence (index ≥ 60 and < 80), which did not occur in CRT. This study revealed a significant improvement for management of pain, vitality, social functioning and mental health domains in SHI as compared to its control. Barthel index demonstrated that SHI is functionally more independent than the CRT. It may be concluded that shiatsu associated with conventional kinesiotherapy in post-stroke patients promotes recovery of functional capacity as well as their quality of life.

Disclosures: H.C. Marcusso: None. **S.M. Tagami:** None. **D. Parizotto:** None. **D.M. dos Santos:** None. **R.N. Isayama:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.12/N5

Topic: C.21.Stroke Recovery

Support: NIH/NINDS R21NS064185

NIH/NINDS R01NS082225

UNM Behavioral Health BBI/CoBRE Res. Award.

Title: *In vivo* inhibition of mir-155 recovery following experimental mouse stroke

Authors: *E. CABALLERO-GARRIDO[1], J. PENA-PHILIPPIDES[1], T. LORDKIPANIDZE[2], D. BRAGIN[1], Y. YANG[3], T. ROITBAK[1];
[1]Neurosurg., Univ. of New Mexico, Albuquerque, NM; [2]Ilia State Univ., Tbilisi, Georgia; [3]BRaIN Imaging Ctr. and Col. of Pharm., , Univ. of New Mexico,, Albuquerque, NM

Abstract: A multi-functional microRNA miR-155 has been recognized as an important modulator of numerous biological and pathological processes such as hematopoietic lineage differentiation, immunity, inflammation, cancer, and cardiovascular diseases. The goal of the present study is to explore the role of miR-155 in regulating cerebral vasculature after stroke. Specifically, we investigated the effect of *in vivo* inhibition of miR-155 on brain microvasculature, as well as infarct size and overall recovery following experimental cerebral ischemia. The intravenous injections of a specific miR-155 inhibitor were initiated at 48 hours after mouse distal middle cerebral artery occlusion (dMCAO), and resulted in 50% inhibition of miR-155 in the brain tissue. The efficiency of *in vivo* miR-155 inihibition was evaluated using

confocal microscopy, PCR and western blot analyses. Microvasculature in peri-infarct area, infarct size and animal functional recovery were assessed at 1, 2 and 3 weeks after dMCAO. Using *in vivo* two-photon microscopy, we detected improved blood flow and microvascular integrity in the peri-infarct area of miR-155 inhibitor-injected mice. Electron microscopy revealed that, in contrast with the control group, these animals had well-preserved capillary tight junctions (TJ). Western blot analysis showed that among all TJ proteins, ZO-1 was consistently affected by miR-155 inhibition. We propose that stabilization of this scaffolding and signaling protein could strengthen TJ barrier function in the inhibitor-injected animals. The improved vascular integrity in these animals was associated with reduced brain damage: MRI analysis showed significant (34%) reduction of infarct size in miR-155 inhibitor-injected mice at 21 days after dMCAO. Reduced brain injury was confirmed by electron microscopy, demonstrating decreased neuronal damage in the peri-infarct area of stroke. Preservation of the brain tissue was reflected in efficient functional recovery of inhibitor-injected animals. Our studies include recovery and behavioral assessment using the "Adhesive Removal," and "CatWalk" test. Based on our findings, we propose that *in vivo* miR-155 inhibition following ischemia supports brain microvasculature, reduces brain tissue damage, and improves the animal functional recovery. This approach could be further explored as a potential treatment for stroke.

Disclosures: E. Caballero-Garrido: None. **J. Pena-Philippides:** None. **T. Lordkipanidze:** None. **D. Bragin:** None. **Y. Yang:** None. **T. Roitbak:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.13/N6

Topic: C.21.Stroke Recovery

Support: Boston Children's Hospital

Georgetown University

MedStar National Rehabilitation Hospital

Title: Lifting brakes on stroke recovery: motor plasticity in Lynx1 knockout mice

Authors: *N. W. HODGSON[1,2], E. NEWPORT[3], A. DROMERICK[3], T. K. HENSCH[1,2]; [1]Boston Children's Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]Georgetown Univ. Ctr. for Brain Plasticity & Recovery, Washington, DC

Abstract: Motor function recovery following stroke is associated with increased plasticity in peri- and contralesional cortex, however this is limited in the adult CNS. Here, we test whether removal of a molecular "brake" on plasticity holds therapeutic value to improve recovery from

stroke. Lynx1 is an endogenous prototoxin similar to the snake venom α-bungarotoxin. It acts to limit cortical rewiring in adulthood by dampening nicotinic acetylcholine receptor function, and mice lacking Lynx1 recover from amblyopia ('lazy eye') long after corresponding wild-type animals have stabilized (Morishita et al., 2010). Using a photothrombotic ischemic cortical injury model in C57BL/6J (WT) and Lynx1 knockout (KO) mice, we compared their respective post-stroke recovery trajectory. Mature male WT and Lynx1 KO mice at both postnatal day 60 (P60) and P300 were trained to proficiency on a skilled capellini handling task, then received ischemic unilateral sensorimotor cortex lesions contralateral to their dominant forepaw. Motor function was assessed by capellini handling as well as DigiGait analysis, and sensorimotor function was measured by an adhesive tape removal task, for 21 days following cortical lesion. Behaviorally, Lynx1 KO mice showed less initial impairment on all tasks and a greater recovery on those associated with motor function. At P60, the amount of time to manipulate and consume a segment of capellini improved faster in Lynx1 KO versus WT controls (τ = 4.942 and 37.87 days, respectively). Recovery rates for other metrics measured in the capellini handling task, tape removal task and gait analysis were similar between groups. Over the same time period, biochemical markers of oxidative stress (GSH/GSSG) were compared across hemispheres. Prior to stroke, P60 Lynx1 KO mice showed a 23% baseline reduction in GSH/GSSG ratio, but were able to recover faster post-stroke than in WT controls (τ = 5.838 and 75.06 days, respectively). Similarly, P300 Lynx1 KO mice showed a 45% baseline reduction in GSH/GSSG ratio and again recovered faster than WT (τ = 8.17 and 34.55 days, respectively). The pre-stroke oxidative shift in GSH/GSSG ratio observed in Lynx1 KO animals may prime antioxidant systems to increase the rate of recovery. Lifting other molecular brakes based on developmental mechanisms, such as axonal growth inhibitors (NgR/PirB signaling; Fang et al 2010, Adelson et al 2012) or extra-cellular matrix components (CSPGs; Gherardini et al 2015) may be similarly beneficial for stroke recovery in adulthood, yet Lynx1 offers a non-invasive strategy via nicotinic receptor signaling.

Disclosures: **N.W. Hodgson:** None. **E. Newport:** None. **A. Dromerick:** None. **T.K. Hensch:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.14/N7

Topic: C.21.Stroke Recovery

Support: R01HD059783

Title: Training of the non-paretic arm in unilateral stroke improves arm function and performance

Authors: *C. CHOPICK[1,2], D. C. GOOD[1], C. WINSTEIN[3], R. L. SAINBURG[1,2];
[1]Neurol., Penn State Col. of Med., Hershey, PA; [2]Dept. of Kinesiology, Penn State Univ., University Park, PA; [3]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: We previously elaborated hemisphere specific motor deficits in the non-paretic, ipsilesional arm of chronic unilateral stroke patients. We now show that these deficits can be associated with substantial limitations in functional performance, especially in patients with moderate to severe contralesional paresis. In this pilot feasibility study, we ask whether intense training of the ipsilesional arm can lead to substantial and durable improvements in functional performance. Non-paretic arm training employed both virtual reality (VR) tasks and challenging real-life activities involving rapid and accurate object placement, object manipulation, tracing, and targeted throwing tasks. Three patients with moderate to severe paresis engaged in a 4 week intervention, involving 3 sessions per week, which lasted for 2 hours each. For 30 minutes, patients focused on VR activities involving rapid accurate motions of the arm, followed by real-life activities for the remaining 1.5 hours. Dependent measures included: 1) Jebsen-Taylor Hand Function Test (JTHFT), 2) Slotted Pegboard test, 3) Kinematics during a center out reaching task, and 4) a modified version of the Functional Independence Measure (FIM). These tests were given twice prior to training, separated by a 1 week interval, immediately following training, and 1 month after training. Improvements were made on measures of unilateral function, activities of daily living, and functional independence, including greater than 15% improvement on the JTHFT, a test of unimanual functional performance, and in reaching kinematics, including improved speed, accuracy, and smoothness. All patients also reported improved functional independence, including one patient reporting that he became completely independent in dressing and undressing and another stating that he now can independently dispense his own toothpaste, and brush his teeth. Our findings provide preliminary support to the idea that short term training of the non-paretic arm can lead to substantial and durable improvements in functional performance.

Disclosures: C. Chopick: None. **D.C. Good:** None. **C. Winstein:** None. **R.L. Sainburg:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.15/N8

Topic: C.21.Stroke Recovery

Support: AHA #14CRP19880025

NIDRR H133P100014

Title: Reactive and voluntary stepping in individuals with stroke: a comparison between paretic and nonparetic leg responses

Authors: C.-L. YANG[1], V. GRAY[1], M. FUJIMOTO[2], *S. MCCOMBE WALLER[1], M. W. ROGERS[1];
[1]Dept. of Physical Therapy and Rehabil. Sci., Univ. of Maryland, Sch. of Med., Baltimore, MD;
[2]Dept. of Sport and Hlth. Sci., Ritsumeikan Univ., Kusatsu, Japan

Abstract: Background: Fall risk after stroke is a major healthcare concern. Impaired lateral weight transfer between paretic (P) and nonparetic (NP) legs during reactive and voluntary stepping may disrupt balance stability and increase fall risk after stroke. Objective: We compared the stepping responses of the P and NP leg during unexpected waist-pulls to the P and NP side versus cued voluntary stepping of the P and NP leg in individuals with chronic stroke. Methods: Fourteen community dwelling individuals >6 months post stroke completed reactive and voluntary lateral step testing. For reactive lateral stepping, 24 trials of randomly-ordered unexpected waist-pull perturbations were applied to the subject (2 directions × 3 repetitions × 4 magnitudes). For voluntary lateral stepping, subjects were instructed to perform 10 trials of a single lateral step as fast as possible according to the direction of a light cue (2 directions × 5 repetitions). The main outcome measures were first step characteristics including step onset, step duration, normalized step clearance, normalized step length in the mediolateral (ML) and anteroposterior (AP) direction, normalized global step length, and normalized center of pressure (COP) velocity. Nonparametric tests were used for comparison of significance between the P and NP leg responses during reactive and voluntary stepping. Results: During reactive stepping, subjects initiated steps with the NP leg 61 % of the time regardless of the pull direction. Compared to the lateral steps initiated with the NP leg, the P leg steps had a marginally lower clearance (P=0.068), longer ML step length (P=0.068), and shorter global step length (P=0.068). In contrast, during voluntary stepping, all subjects could generate a P voluntary lateral step, however step clearance (P=0.056), ML step length (P=0.026), and global step length were smaller (P=0.056). The AP step length was larger compared to NP stepping (P=0.011). Across the four conditions (reactive lateral steps initiated with P/NP leg and voluntary lateral steps initiated with P/NP leg), a significant difference was found in the first step duration (P=0.031). Post hoc analysis showed a significantly longer step duration in voluntary NP leg stepping compared to reactive NP leg stepping (P=0.046). Conclusion: We have identified differences between P and NP leg responses during reactive and voluntary stepping in individuals post-stroke. This has implications for the development of rehabilitation interventions to prevent falls in this high risk group.

Disclosures: C. Yang: None. **V. Gray:** None. **M. Fujimoto:** None. **S. McCombe Waller:** None. **M.W. Rogers:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.16/N9

Topic: C.21.Stroke Recovery

Support: DHSA Pilot Grant

NIH R01NR010786

Title: Asymmetrical corticomotor input to the plantarflexors influences the biomechanical strategy of speed modulation in individuals post-stroke

Authors: *J. A. PALMER[1], H. HSIAO[2], L. N. AWAD[4], S. A. BINDER-MACLEOD[3]; [2]Biomechanics and Movement Sci., [3]Physical Therapy, [1]Univ. of Delaware, Newark, DE; [4]Sch. of Engin. and Applied Sci., Harvard Univ., Boston, MA

Abstract: In the presence of lost function following brain injury, one of the most common and consistent observations is that individuals develop compensation strategies and demonstrate heavy reliance on the nonparetic limb during gait. Such compensations have been shown to be related to major neuronal reconstruction and growth in the cerebral cortex, which can translate to cortical imbalances between the lesioned and nonlesioned hemisphere related to poor motor recovery. Impaired ankle moment generation of the paretic limb has been identified as a significant contributor to walking-related disability after stroke and is related to walking speed. However, when individuals post-stroke are asked to increase walking speed (i.e. modulate gait speed), different biomechanical strategies are used that are independent of their level of walking function. We hypothesized that balance of contralateral corticomotor input to the paretic and nonparetic plantarflexor muscles (i.e. corticomotor asymmetry) will moderate the relationship between change in paretic ankle moment and change in gait speed. To test this hypothesis, we measured corticomotor input to the paretic and nonparetic soleus muscles and the paretic ankle moments at self-selected and fast walking conditions in 19 persons with hemiparesis following stroke. Preliminary results indicated that no relationship exists between magnitude of gait speed modulation versus changes in paretic ankle moment during speed modulation ($r=0.035$) ($p=0.44$) or corticomotor asymmetry ($r=0.122$) ($p=0.30$). However, there was a strong relationship between change in paretic ankle moment with speed modulation and corticomotor asymmetry ($r=0.629$) ($p<0.01$). This provides novel evidence that asymmetrical corticomotor input to the lower extremity may underlie biomechanical compensation strategies used for gait speed modulation post-stroke. These findings have significant implications for approaches used in post-stroke rehabilitation.

Disclosures: J.A. Palmer: None. **H. Hsiao:** None. **L.N. Awad:** None. **S.A. Binder-Macleod:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.17/N10

Topic: C.21.Stroke Recovery

Support: Consejo Nacional de Ciencia y Tecnología, México Grant: 219542

Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica DGAPA-UNAM. Grant: IA201315

IBRO Return Home Program grant to LBTyR

Title: Astrocyte-derived exosomes reduce infract volume and reduce neurological alterations in a rat model of ischemic stroke

Authors: B. BERNAL-VICENTE, E. HERNÁNDEZ-PONCE, A. RAMOS-MORALES, *L. B. TOVAR Y ROMO;
Univ. Nacional Autonoma de Mexico, Mexico City, Mexico

Abstract: Stroke is the third cause of death and permanent disabilities worldwide. Given the lack of effective therapies for this type of acute insults to the CNS, it is important to understand how endogenous mechanisms for brain repair activated upon ischemia modulate the outcome and promote tissue recovery. One of the main mediators of the endogenous response elicited after stroke is vascular endothelial growth factor (VEGF), which is secreted by astrocytes and has been shown to reduce infarct volume in experimental models *in vivo* when administered exogenously. Here, we explored how astrocytes contribute to protect neurons after ischemia by releasing exosomes containing signaling mediators of survival and whether this effect is dependent on VEGF-mediated signaling. For this, we cultured rat primary cortical astrocytes that were subjected to hypoxia for 2 hours. Then, exosomes released over a period of 24 hours were collected and administered by stereotaxic intraventricular injection to rats subjected to transient middle cerebral artery occlusion (MCAO) 30 min after reperfusion. We found that astrocyte-derived exosomes reduced the infarct volume, as assessed by tretazolium chloride staining, and reduced the neurological symptoms manifested 24 hours after stroke. We then investigated whether the protection was driven by the activation of VEGF receptor 2 and found that this receptor was partially involved in the neuroprotection. We also studied the effect of astrocyte-derived exosomes on the modulation of blood brain barrier permeability, which is known to play an important role in the development of the neuroinflammatory process that occurs after stroke. We found that exosomes reduced the amount of protein extravasation induced by stroke, which might also constitute a mechanism for astrocyte-mediated neuroprotection. These results are important clues to understand the mechanisms of intercellular communication that participate in the concerted response that takes place in the brain after an acute insult and may point to new therapeutic targets that could be potentiated by clinical interventions.

Disclosures: B. Bernal-Vicente: None. **E. Hernández-Ponce:** None. **A. Ramos-Morales:** None. **L.B. Tovar y Romo:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.18/N11

Topic: C.21.Stroke Recovery

Support: CTI Grant #14668

Title: Virtual reality based upper limb neurorehabilitation in acute stroke: a single-case study

Authors: H. KINZNER[1], *G. GARIPELLI[2], D. PEREZ-MARCOS[2], T. TADI[2], K. DISERENS[1];
[1]Dept. of Clin. Neurosciences, Ctr. Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [2]MindMaze SA, Ecublens, Switzerland

Abstract: A 60-year-old right-handed (Edinburgh Test > 95) male subject was treated at the acute Neurorehabilitation unit at the Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland) with a Virtual Reality (VR) based upper limb motor rehabilitation system (MindMotionPRO, MindMaze SA), starting 9 days post stroke. Pre-intervention medical evaluation revealed ischemic stroke (left paramedian pontine), resulting in hemiparesis on the right side (on day 8, NIHSS = 6; Frenchay Arm Test score = 3/5; Fugl-Meyer Assessment Upper Extremity (FMA-UE): synergy & reflex of shoulder, elbow and forearm score = 26/66, joint pain score = 21/24, sensation score = 8/12 and passive joint motion score = 22/24). The patient carried out VR reaching exercises implying active shoulder and arm movements for approximately 1 hour per day, using two visual feedback mechanisms: (i) direct mode (patient arm movements are translated to the ipsilateral avatar arm); and (ii) mirror mode (patient arm movements are translated to the contralateral avatar arm), resulting in a “virtual mirror illusion”. The mirror mode is similar to mirror therapy and aims at activating the neural pathways ipsilateral to stroke lesion. It was applied when the patient reported fatigue of the paretic arm. The patient completed 3 sessions in 3 consecutive days, comprising a total of ~400 reach movements. Exercises were undertaken in a game-like scenario that enhanced patient motivation. During his stay in the acute care, the patient also undertook thrombolysis treatment and nearly 1 hour of physical therapy per day. On the day of discharge (day 13; NIHSS = 3) there was an improvement in arm synergy (FMA-UE synergy & reflex of shoulder, elbow and forearm score = 32/66) and a reduction in the joint pain (joint pain score = 24/24). There were no pre-post changes in the wrist and hand movement score, sensation score or functional skills (as indicated by the Frenchay Arm Test). Overall, the subject was highly motivated and immersed in the VR tasks provided. From the results of this study, we surmise that the administration of VR based upper limb motor rehabilitation in the acute care is feasible and well accepted by the patient. Emerging evidence suggests that such training in the early phases of acute stroke recovery results in significant improvements in brain activation and functional outcomes. We believe that the

virtual mirror training can activate the neural mechanisms underlying motor recovery even when the patients have a very low motor function of the paretic arm.

Disclosures: H. Kinzner: None. **G. Garipelli:** None. **D. Perez-Marcos:** None. **T. Tadi:** None. **K. Diserens:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.19/N12

Topic: C.21.Stroke Recovery

Support: Governors State University Research Grant to RDT

Governors State University Research Grant to RKO

Title: Impact of a Conductive Education intervention on supraspinal structures in adults with chronic stroke

Authors: *R. D. THEISS[1], T. B. PARRISH[2], R. K. O'SHEA[1];
[1]Physical Therapy, Governors State Univ., University Park, IL; [2]Radiology, Northwestern Univ., Chicago, IL

Abstract: The location and severity of damage to the brain after a stroke influences the extent of functional limitations experienced by the stroke survivor. After injury, measurable physiological changes continue that can be correlated with functional clinical measures. With physical rehabilitation interventions, functional impairments can be lessened, presumably through mechanisms of neuroplasticity. Though interventions are often effective for restoring at least partial function for individuals with stroke, little is known about what underlies the positive results for specific interventions. The purpose of this study was to examine the connections within the brain of stroke survivors who have undergone physical rehabilitation but still have persisting physical impairments and assess changes in neurological functional and structural connectivity after participating in a novel cognitive-physical rehabilitation intervention program known as Conductive Education (CE). CE is a multidisciplinary, motor-learning based intervention. This study expanded the use to adults with chronic stroke and added imaging. Four adults with chronic (>6mo) stroke were enrolled into the study. All subjects had completed outpatient rehabilitation at least 5yrs prior. Two subjects had pontine-level lesions; two had subcortical lesions. Prior to CE participation, baseline measures of function (via clinical assessment) and supraspinal neurological structures (via imaging) were obtained. A licensed physical therapist evaluated each subject for measures of physical impairment and social and community participation. Additionally, Magnetic Resonance Imaging (MRI) data were collected to assess functional and structural connectivity, myelin concentration, and cerebral perfusion.

This imaging included resting fMRI, diffusion tensor imaging, and an arterial spin labeling method. Subjects then participated in weekly, 2-hour CE program sessions for 10 weeks. At the end of the CE program, clinical assessment measures and imaging data were again collected and analyzed for pre/post intervention changes. Preliminary results showed that participants improved physical function and performance as well as community participation at or above the levels of minimal clinical important difference (MCID). The improvements in hand function were statistically significant within the group, but other improvements were individual and varied. Likewise, varied imaging results are expected. From this pilot study, we conclude that a CE intervention could be effective for adults with chronic stroke by inducing supraspinal changes measurable with emerging imaging techniques.

Disclosures: R.D. Theiss: None. **T.B. Parrish:** None. **R.K. O'Shea:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.20/N13

Topic: C.21.Stroke Recovery

Support: NINDS, NIH: 1 R15 NS084130-01A1

Title: Changes in motor unit firing behavior following maximal efforts post stroke

Authors: *K. BATHON[1], T. ONUSHKO[2], J. NGUYEN[3], E. MCGONIGLE[4], N. GEOFFROY[3], N. KETCHUM[4], F. NEGRO[5], D. FARINA[5], S. HUNTER[3], B. SCHMIT[2], A. HYNGSTROM[3]; [1]Clin. and Translational Rehabil., [2]Biomed. Engin., [3]Physical Therapy, Marquette Univ., Milwaukee, WI; [4]Physical Med. and Rehabil., Med. Col. of Wisconsin, Milwaukee, WI; [5]Neurorehabilitation Engin., Goettingen Georg-Augst Univ., Goettingen, Germany

Abstract: Motor unit firing behavior is altered post stroke and may contribute to force generating impairments. In particular, there is saturation in firing rates as compared to firing rates in healthy controls. It is not known if paretic motor unit firing behaviors can modulate in response to excitatory inputs. The purpose of this study was to quantify the effects of maximal efforts on knee extensor motor unit discharge rates in chronic stroke. Twelve chronic stroke subjects (63 ± 6 years old) and ten control subjects (62 ± 5 years old) participated in this study. Knee extension torque was recorded using a Biodex dynamometer equipped with a JR3 load cell. Vastus lateralis motor unit discharge rates were extracted from surface motor unit potentials recorded using a high-density surface array (stroke n = 33 units, control = 47 units). Baseline maximal voluntary contractions (MVCs) of the knee extensors were recorded for the paretic leg of stroke subjects (PL) and the dominant leg of control subjects (CL). Following the MVCs, subjects practiced producing 30% knee extension MVC torques while they were provided with

visual feedback of their performance. Then, visual feedback was removed and subjects performed sub-maximal contractions under two different conditions (randomized): (A) a set of three 5 s contractions each followed by 10 s rest at 30% MVC (repeated three times with 30 s rest between each set) and (B) the same as protocol A, but with the addition of an MVC between each of the 5 s contractions. Finally, subjects were asked to produce what they believed to be 30% of their knee extension MVC. There was a significant increase in knee extension torque in the PL during the sub-maximal portion of Protocol B compared with Protocol A (% MVC ± SE: 31% ± 3% vs. 23% ± 3%, t-test, $p < 0.05$), while there was no significant change in torque production observed for the CL (34% ± 1% vs. 31% ± 2%, t-test, $p > 0.05$). Stroke and control had increased firing rates at recruitment during sub-maximal contractions for Protocol B (stroke = 9.8pps ± 1.1; control 12.4pps ± 1.7) as compared to Protocol A (stroke = 6.5pps ± 0.7, control = 9.1pps ± 0.7). In addition, for the stroke group, peak firing rates during Protocol B (7.6pps ± 0.8) were greater as compared to Protocol A (5.9pps ± 0.5). On average, the mean firing rates during contractions for Protocol B (stroke = 9.6 ± 0.7; control = 12.8 ± 0.8) were greater for both groups as compared to Protocol A (stroke = 8.5 ± 0.5; control = 11.8 ± 0.7). These data suggest that immediately following a maximal effort, paretic motor units can modulate firing rates. The change in firing rates is likely due to additional neural drive to the motorneuron pools from the brief maximal contraction.

Disclosures: **K. Bathon:** None. **T. Onushko:** None. **J. Nguyen:** None. **E. McGonigle:** None. **N. Geoffroy:** None. **N. Ketchum:** None. **F. Negro:** None. **D. Farina:** None. **S. Hunter:** None. **B. Schmit:** None. **A. Hyngstrom:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.21/N14

Topic: C.21.Stroke Recovery

Support: Swiss National Science Foundation NCCR on Neural Plasticity and Repair

EMDO Foundation

Title: Brain reorganisation following robot-assisted therapy in stroke patients

Authors: ***N. ESTÉVEZ**[1], V. KLAMROTH-MARGANSKA[2,3], B. HARTOG-KEISKER[1], L. MICHELS[1], M.-C. HEPP-REYMOND[4], R. RIENER[2,3], S. KOLLIAS[1];
[1]Dept. of Neuroradiology, Univ. Hosp., Zurich, Switzerland; [2]Sensory-Motor Systems Lab., ETH Zurich, Switzerland; [3]Univ. Hosp. Balgrist, Zurich, Switzerland; [4]Inst. of Neuroinformatics, University of Zurich and ETH Zurich, Switzerland

Abstract: Stroke often leads to chronic motor disability of the upper limb, which can severely affect patients' activities of daily living. Recovery of function following a stroke is related to brain reorganisation, which can be promoted by movement therapy. Additionally, previous evidence indicates that robot-assisted therapies providing intensive and task-specific training of the affected limb may be particularly effective at restoring motor function. In the present study, we investigated brain reorganisation induced by robotic therapy in patients with chronic stroke. Patients suffering from moderate and severe upper limb impairments were trained with ARMin - an exoskeleton robot, which supports movements of the entire limb and allows task-specific training in the three dimensional spaces. To assess therapy-induced changes in brain activation, functional magnetic resonance imaging (fMRI) was performed during repetitive active and passive elbow flexion/extension movements at three time points: before therapy, after eight weeks of movement therapy, and at two-month follow-up. To provide constant and accurate performance of the tasks across sessions, an MRI-compatible robot guided and monitored movements during recordings. Additionally, data from several behavioural measures, assessed at each time point, were included to evaluate motor performance over time. This study includes data from eight patients with moderate and severe motor impairment. After therapy with ARMin gains in motor performance were observed in all trained patients. At two-month follow-up, performance remained stable and even slightly improved in patients with moderate deficits, whereas the outcome of the severely impaired patients was more variable. Training led to reduced activation volume in the sensorimotor cortex for passive and active movements in almost all moderately impaired patients. Most patients suffering from severe impairment showed an increase in activation in the sensorimotor cortex during active tasks. For passive movements, no activation was found for half of the severely impaired patients during all three assessments. The remaining patients also showed a tendency for increased activation. Changes observed after therapy often persisted at two-month follow-up, especially in moderately impaired during both tasks and severely impaired during active ones, though they were less pronounced. Our results demonstrated that robot-assisted therapy promotes brain reorganisation and functional arm recovery in patients with chronic stroke. However, the induced reorganisation patterns and long-term effects of the therapy depend on the degree of impairment.

Disclosures: **N. Estévez:** None. **V. Klamroth-Marganska:** None. **B. Hartog-Keisker:** None. **L. Michels:** None. **M. Hepp-Reymond:** None. **R. Riener:** None. **S. Kollias:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.22/N15

Topic: C.21.Stroke Recovery

Support: CIHR MOP126198

Heart and Stroke Foundation

Title: Assessing cognitive function following medial prefrontal stroke in the rat

Authors: *J. LIVINGSTON-THOMAS[1,2], M. JEFFERS[1,2], C. NGUEMENI[1,2], M. SHOICHET[3], C. MORSHEAD[3], D. CORBETT[1,2,4];
[1]Univ. of Ottawa, Ottawa, ON, Canada; [2]Canadian Partnership for Stroke Recovery, Ottawa, ON, Canada; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Mem. Univ., St. John's, NL, Canada

Abstract: Cognitive impairments are prevalent following clinical stroke. However, to date, preclinical research has focused almost exclusively on motor deficits. In order to conduct systematic evaluations into the nature of post-stroke cognitive dysfunction and recovery, it is crucial to develop focal stroke models that affect cognition while leaving motor function intact. Furthermore, in order to investigate potential cognitive post-stroke treatments, it is important that deficits are persistent in the chronic phase. This experiment was performed to evaluate a focal medial prefrontal cortex (mPFC) stroke model using a battery of tests that examined a range of cognitive functions 1-4 months following stroke. Male Sprague-Dawley rats weighing 250-300 g underwent focal ischemia induced in the mPFC using bilateral intracerebral injections of endothelin-1, or sham surgery. Beginning at 1 month post-stroke, cognitive function was assessed using open field, temporal object recognition, object-context recognition, object-placement recognition, attentional set-shifting, light-dark box, spontaneous alternation, Barnes maze, and win-shift/win-stay tests. Prefrontal cortex injury resulted in bilateral damage to the prelimbic and cingulate cortices, extending typically between 4.22 to 1.34 mm anterior to bregma. Animals that underwent stroke surgery exhibited significant changes in all object recognition functions compared to Sham animals ($p<0.05$). Stroke animals also exhibited impaired performance on the Barnes maze ($p=0.012$), and took significantly more trials to learn the second rule in the win-shift/win-stay test ($p=0.013$). Further, they exhibited reduced anxiety-like behaviour in the open field ($p=0.049$). Spontaneous alternation behaviour and locomotion in the open field were not affected. The deficits observed are consistent with some of the key characteristics of prefrontal stroke in humans. Our results show that this model produces persistent deficits in multiple prefrontal cognitive functions, and therefore may be useful for identifying and developing potential therapies for improving cognitive dysfunction in the chronic phase following stroke.

Disclosures: J. Livingston-Thomas: None. **M. Jeffers:** None. **C. Nguemeni:** None. **M. Shoichet:** None. **C. Morshead:** None. **D. Corbett:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.23/N16

Topic: C.21.Stroke Recovery

Support: American Brain Foundation CRFT grant

Title: Electrically preconditioned neural progenitor cells on a conductive polymer scaffold enhance stroke recovery

Authors: *P. GEORGE, T. M. BLISS, T. HUA, A. LEE, S. MEHTA, G. SUN, G. K. STEINBERG;
Neurol., Stanford Univ., Stanford, CA

Abstract: Background: Human neural progenitor cells (hNPCs) improve functional recovery after stroke in pre-clinical models. However, the optimal conditions for delivery and exact pathways of recovery remain elusive. Tissue engineered scaffolds offer a unique method to manipulate the environment for hNPC transplantation. We have developed a novel hNPC delivery system utilizing an electrically conductive polymer scaffold to improve recovery and further elucidate repair mechanisms. Methods: Human hNPCs were seeded onto an electrically conductive polymer scaffold. Electrical fields were applied to the hNPCs to electrically "precondition" the cells. One day after *in vitro* stimulation, the polymer scaffold was implanted into a distal MCA occlusion rat model 1 week post-stroke. Subsequently, functional recovery was assessed using the vibrissae-paw test and neurological scores. Control groups consisted of unstimulated hNPCs on the polymer and polymer alone without cells. To assess how electrical stimulation altered the cells, we performed qPCR analysis of genes of interest in stimulated and unstimulated cells on the polymer, and in hNPCs on a plastic petri dish. To further elucidate mechanisms, VEGF-A was blocked with bevacizumab during the *in vitro* preconditioning period. Results: Alternating current (AC) preconditioned hNPCs improved the rate of neurologic functional recovery compared to unstimulated hNPCs on the polymer ($p<0.05$) and significantly improved recovery compared to direct current (DC) preconditioned cells and polymer alone ($p<0.01$). VEGF-A was upregulated in AC preconditioned cells compared to other groups ($p<0.05$). If VEGF-A was inhibited during electrical preconditioning, no improvement in behavior was observed compared to the unstimulated hNPCs. Blood vessel density also increases with electrically preconditioned hNPCs ($p<0.05$) and returns to baseline if VEGF-A is blocked. Conclusions: Our results show that a conductive polymer scaffold can be used to enhance stroke recovery and manipulate hNPCs to elucidate mechanisms of stroke recovery. VEGF-A (a protein critical to angiogenesis and other plasticity processes) was found to be modified by electrical preconditioning of hNPCs, suggesting one possible mechanisms for enhanced functional recovery.

Disclosures: P. George: None. **T.M. Bliss:** None. **T. Hua:** None. **A. Lee:** None. **S. Mehta:** None. **G. Sun:** None. **G.K. Steinberg:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.24/N17

Topic: C.21.Stroke Recovery

Support: NIH Grants R01 NS062097, R01 NS058710, R01 NS085568

Title: Whisker stimulation enhances oligodendrogenesis and axonal/dendritic repair in the barrel cortex following focal ischemia in mice

Authors: *J. SUN[1,2,3], J. LEE[2], X. GU[2], Z. Z. WEI[3], Y. ZHANG[3], J. LI[3], S. YU[2], L. WEI[2]; [1]Labs of Stem Cell Biol and Regenerative Med., Beijing, China; [2]Anesthesiol. and Neurol., Emory Univ. Sch. of Med., Atlanta, GA; [3]Neurol., Beijing Friendship Hosp. Capital Med. Univ., Beijing, GA

Abstract: Peripheral stimulation and activity have significant 'use-dependent' impacts on morphological and functional alterations in the central nervous system (CNS) and on the outcome of CNS disorders. Our previous studies showed that stimulating corresponding whiskers after focal cerebral ischemia in the whisker-barrel cortex promoted neurogenic and angiogenic activities and functional recovery. In the present study, we tested the hypothesis that enhancing peripheral activity and sensory input to the ischemic barrel cortex by whisker stimulation might promote oligodendrogenic activity and axonal/dendritic repair in the ischemic barrel cortex of mice. We used a nestin-CreER/YFP transgenic mouse for neural cell lineage tracing. Three days after focal barrel cortex ischemia in mice, whisker stimulations were manually performed (15 min × 2 times/day) to enhance afferent signals to the ischemic barrel cortex. By 14 days after stroke, significantly more nestin-CreER/YFP+ cells in whisker stimulation group were evident in the subventricular zone (SVZ), corpus callosum, and penumbra regions. Whisker stimulation group exhibited significant increases of YFP+/DCX+ and YFP+/Oligo2+ cells while there was no change of YFP+/GFAP+ cells, indicating enhanced neurogenesis and oligodendrogenesis, but not astrocytogenesis. Immunostaining and Western blot analyses revealed that the ischemica-induced reductions of neurofilament and myelin basic protein were significantly recovered by whisker stimulation. In addition, whisker stimulation fully prevented the reduction in the expression of synapsin-1, synaptophysin, and growth-associated protein-43 (GAP-43). Whisker stimulation also improved functional recovery tested using adhesive removal test and HomeCage monitoring system. Our data suggest that peripheral stimulation can increase regenerative activities including oligodendrogenesis, enhance axonal/dendritic repair, and prevent synaptic damages in the post-ischemic brain, which may contribute to long-term functional recovery from ischemic stroke.

Disclosures: **J. Sun:** None. **J. Lee:** None. **X. Gu:** None. **Z.Z. Wei:** None. **Y. Zhang:** None. **J. Li:** None. **S. Yu:** None. **L. Wei:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.25/N18

Topic: C.21.Stroke Recovery

Support: The Richard Merkin Foundation for Neural Regeneration at UCLA

Dr. Miriam & Sheldon G. Adelson Medical Research Foundation

Title: CREB/DREADD system: switching on/off recovery of motor function after stroke

Authors: *L. CARACCIOLO[1], A. HAMADE[2], T. BULFONE[2], A. GUZNER[2], Y. SANO[3], A. J. SILVA[3], S. T. CARMICHAEL[2];
[1]Neurol., David Geffen Sch. of Med. At UCLA, Los Angeles, CA; [2]Neurol., [3]Neurobio., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: CREB plays a major role in learning, neuronal plasticity and memory formation. We investigated the role of CREB in promoting neuronal recovery after focal stroke. This was done by promoting a gain and/or a selective loss of function in delivery of CREB in the peri-lesion motor cortex using inhibitory hM4Di DREADD lentivirus(Designer Receptors Exclusively Activated by Designer Drugs) which is coupled to Gi signaling and activates the inward rectifying potassium channel that inhibits neuronal excitability. Mice received an injection of lentivirus that over-express CREB+hM4Di or hM4Di alone at the time of stroke. Mice were tested behaviorally over 12 weeks after stroke. Saline or Clozapine-N-Oxide (CNO, an inert molecule), that stimulates DREADD receptors (selective loss of function) was delivered 30 minutes before each behavioral test. CREB/hM4Di over-expression shows enhanced motor recovery. Mice transfected with CREB/hM4Di + saline have faster speed in eating pasta, reduction in the number of foot faults in grid-walking, and preference for right paw rearing in cylinder task 4 weeks after stroke. Mice transfected with the control virus hM4Di + CNO showed a greater deficit in recovery after stroke for all the behavioral tasks performed during the entire treatment. Unexpectedly, the administration of CNO to CREB/hM4Di transfected mice showed reduced motor performance after stroke, even worse than the control virus in stroke (hM4Di + stroke + CNO). Moreover, we observed motor deficit in non-stroke mice with CREB/hM4Di + CNO was comparable to the stroked mice with the control virus (hM4Di + stroke + CNO). In conclusion, CREB induction in a small pool of cells of forelimb motor cortex promotes recovery. Inhibition of neuronal activity in this small pool of cells (hM4Di + CNO) doesn't have any effect on motor performance (control mice). Surprisingly, inhibition of neuronal activity in CREB-transfected neurons causes degraded motor control, worse than the effects observed in stroked mice transfected with the control virus (hM4Di + stroke + CNO). This is also seen in CREB/hM4Di + CNO (non-stroke). Thus inducing CREB in motor cortex promotes a network for recovery after stroke. When this network is acutely inhibited, not only the recovered function in stroke is lost, but overall motor control is degraded. This is seen to a statistically lesser degree even when CREB is induced in normal (non-stroke) motor cortex. The plasticity induced by CREB and stroke enable a local network of neurons to control a large

region of functional motor circuitry. Supported by: The Richard Merkin Foundation for Neural Regeneration at UCLA, Dr. Miriam & Sheldon G. Adelson Medical Research Foundation

Disclosures: **L. Caracciolo:** None. **A. Hamade:** None. **T. Bulfone:** None. **A. Guzner:** None. **Y. Sano:** None. **A.J. Silva:** None. **S.T. Carmichael:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.26/N19

Topic: C.21.Stroke Recovery

Title: Investigating vagus nerve stimulation paired with motor rehabilitation to enhance recovery on a novel task measuring supination and the generalization of recovery across different motor tasks in a rat model of ischemic stroke

Authors: *E. MEYERS[1], S. HAYS[1], R. SOLORZANO[2], R. CHOI[2], M. KILGARD[2], R. RENNAKER[1];
[1]Erik Jonsson Sch. of Engin. and Computer Sci., [2]Sch. of Behavioral and Brain Sci., Univ. of Texas At Dallas, Richardson, TX

Abstract: Stroke affects millions each year, frequently resulting in loss of motor control in the upper extremities. Precise arm and hand function including supination and force generation are commonly affected. Patients often undergo extensive physical rehabilitation but most fail to achieve substantial recovery of function, highlighting the need for improved rehabilitative interventions. Reorganization of neural circuitry throughout the central nervous system is believed to be responsible for recovery and the degree of reorganization is associated with the benefits of rehabilitation. Therapies capable of enhancing reorganization during rehabilitation may enhance functional recovery and improve the quality of life for these patients. By pairing stimulation of the left cervical vagus with motor training, we are able to enhance reorganization in motor circuitry. Consistent with this enhancement of plasticity, we find that vagus nerve stimulation (VNS) paired with rehabilitative training significantly improves recovery of forelimb function in rat models of ischemic and hemorrhagic stroke, traumatic brain injury, and spinal cord injury. In this study we will test if VNS can enhance functional recovery after stroke on a novel task measuring supination, and if this recovery generalizes to a different task measuring forelimb strength. Animals are trained to reach through a small aperture in a clear acrylic cage, grasp a spherical knob, and then supinate to receive a reward pellet. Once the animal trained to the specified degree threshold, a unilateral primary motor cortex ischemic lesion (Endothelin-1) is administered and a vagus nerve cuff implanted. Animals are divided in to two groups: one group receiving VNS paired with rehabilitation on the supination task for 6 weeks, and the other group receiving no stimulation but an identical amount of rehabilitation on the same supination

task. Following the conclusion of rehabilitation, animals are then tested on a separate task measuring volitional forelimb force generation to observe any generalization of recovery across dissimilar tasks. We are currently in the process of evaluating the ability of VNS to improve supination function after stroke. Adult female Sprague-Dawley rats are used and all handling, housing, surgical procedures, and behavioral training of the rats are approved by the University of Texas Institutional Animal Care and Use Committee. The results from this study will shape the design of upcoming stroke clinical trials and provide valuable insight in VNS mediated recovery seen in previous studies.

Disclosures: **E. Meyers:** None. **S. Hays:** None. **R. Solorzano:** None. **R. Choi:** None. **M. Kilgard:** None. **R. Rennaker:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.27/N20

Topic: C.21.Stroke Recovery

Support: NRF-2014R1A2A1A01005128

NRF-2006-2005330

Title: Enhancement of motor recovery using dual-mode noninvasive brain stimulation over the bilateral primary motor cortices in stroke patients

Authors: ***E. PARK**, J. CHO, W. CHANG, A. LEE, Y.-H. KIM;
Samsung Med. Ctr., Seoul, Korea, Republic of

Abstract: Introduction: Noninvasive brain stimulation (NBS) using the repetitive transcranial magnetic stimulation (rTMS) or the transcranial direct current stimulation (tDCS) was recently adopted for modulating motor function of stroke patients. We investigated the effect of simultaneous dual-mode stimulation using rTMS and tDCS over bilateral primary motor cortices (M1) whether it is more effective than single stimulation using rTMS for recovery of motor functions and properties of corticospinal tract (CST) in subacute stroke patients. Methods: Thirty subacute stroke patients whose total Fugl-Meyer Assessment (FMA) score marked under 84 were recruited in this double-blind study. In the dual-mode stimulation group, the 10 Hz rTMS (90% of resting motor threshold, 1,000 pulses) were applied over the ipsilesional M1 for 20 minutes with simultaneous application of the cathodal tDCS (2mA) on the contralesional M1. Single stimulation group underwent 10 Hz rTMS alone without tDCS. Ten daily sessions were conducted for two consecutive weeks. The upper, lower and total FMA scores were measured before, after, and two months after the intervention. To analyze the structural integrity of CST, all patients underwent MRI including diffusion tensor imaging (DTI) at the same time. The

integrity of CST was measured by the ratio of fractional anisotropy (rFA, ipsilesional FA/ contralesional FA). Results: The scores of upper and total FMA scores were significantly improved over time in both the dual-mode and the single stimulation group ($p<0.05$). However, there were significant group and time interaction effects in both upper and total FMA scores ($p<0.05$). Post-hoc analysis showed that the mean changes in upper ($p=0.034$) and total ($p=0.024$) FMA scores were significantly better in the dual-mode stimulation group than the single stimulation group over time. Furthermore, the rFA of CST showed positive correlations with the upper ($r=0539$, $p=0.038$) and total ($r=0548$, $p=0.043$) FMA score at two months after intervention only in the dual-mode stimulation group. Conclusion: The dual-mode NBS with simultaneous application of 10 Hz rTMS and the cathodal tDCS over the bilateral M1s was safe and superior to 10 Hz rTMS alone for enhancing motor recovery and plastic change of CST integrity in subacute stroke patients.

Disclosures: E. Park: None. **J. Cho:** None. **W. Chang:** None. **A. Lee:** None. **Y. Kim:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.28/N21

Topic: C.21.Stroke Recovery

Support: Michael J Fox Foundation

Title: Vagus nerve stimulation paired with rehabilitative training improves recovery of forelimb function in an aged model of ischemic stroke

Authors: *A. D. RUIZ[1], S. A. HAYS[2], D. R. HULSEY[3], N. KHODAPARAST[4], R. L. RENNAKER, II[2], M. P. KILGARD[3];
[1]Texas Biomed. Device Ctr., Univ. of Texas At Dallas, Plano, TX; [2]Dept. of Bioengineering, [3]Brain and Behavioral Sci., [4]Texas Biomed. Device Ctr., Univ. of Texas at Dallas, Richardson, TX

Abstract: Stroke is a debilitating neurological event affecting nearly 7 million people in the U.S. Advanced age is a leading risk factor for stroke and is associated with worse recovery. Following a stroke, many patients are left with some degree of impairment in upper extremity function, even after intensive rehabilitation therapy. Recent studies indicate that vagus nerve stimulation (VNS) paired with rehabilitative training significantly enhances recovery of multiple measures of forelimb strength and movement speed in models of ischemic stroke, intracerebral hemorrhage, and traumatic brain injury. However, all of these studies have been performed in young rats. It is possible that advanced age, which limits neuroplasticity and post-stroke recovery, may occlude the beneficial effect of VNS therapy. To further the translational potential of VNS therapy, we

evaluated the ability of VNS paired rehabilitative training to drive neuroplasticity and improve forelimb recovery in a model of ischemic stroke in aged rats. A cohort of rats, aged 16 months, was trained to perform the isometric force task, an automated and quantitative measure of forelimb function, to proficiency. Following a cortical ischemic lesion, rats underwent rehabilitative training for 6 weeks with or without VNS paired with forelimb movement. Motor maps were derived using intracortical microstimulation (ICMS). Our results indicate that VNS paired with rehabilitative training improves recovery of forelimb strength compared to equivalent rehabilitative training without VNS motor cortex compared to rehab alone. To whether reorganization in circuitry within the motor cortex is enhanced by VNS to support recovery, we are evaluating morphological changes in the perilesional and homotopic contralesionsal motor cortex. Our results indicate that VNS paired with rehabilitative training may effectively enhance recovery in elderly stroke patients.

Disclosures: **A.D. Ruiz:** A. Employment/Salary (full or part-time):; University of Texas at Dallas. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Michael J Fox Foundation. **S.A. Hays:** A. Employment/Salary (full or part-time):; University of Texas at Dallas. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Michael J Fox Foundation. **D.R. Hulsey:** A. Employment/Salary (full or part-time):; University of Texas at Dallas. **N. Khodaparast:** A. Employment/Salary (full or part-time):; University of Texas at Dallas. **R.L. Rennaker:** A. Employment/Salary (full or part-time):; University of Texas at Dallas. **M.P. Kilgard:** A. Employment/Salary (full or part-time):; University of Texas at Dallas.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.29/N22

Topic: C.21.Stroke Recovery

Support: NIH F31 NS086441

NIH R01 NS046400

Title: Overexpression of soluble hemopexin as a therapeutic tool for intracerebral hemorrhage

Authors: ***A. LAMPERT**[1,2], J. L. LECLERC[1,2,3], J. SANTIAGO-MORENO[1,2], A. DANG[1,2], S. DORE[1,2,3,4];
[1]Anesthesiol., [2]Ctr. for Translational Res. in Neurodegenerative Dis., [3]Neurosci., [4]Neurology, Psychiatry, Psychology, and Pharmaceutics, Univ. of Florida, Gainesville, FL

Abstract: Intracerebral hemorrhage (ICH) has the highest morbidity, disability, and mortality rates of any stroke subtype, including ischemic stroke. When a hemorrhagic stroke occurs, the

blood-brain barrier is broken and blood components enter the brain. A major cause of morbidity and mortality following ICH is the direct toxicity of blood metabolites, namely free heme, on adjacent brain tissue. Hemopexin (Hpx) is the endogenous protein responsible for scavenging free heme; thereby, modulating its proxidant and proinflammatory properties. Given the low relative level of Hpx expression in the brain, we hypothesized that overexpression of Hpx would improve anatomical and functional outcomes after ICH. Unique adeno-associated viral vectors (and control vectors) were designed to specifically overexpress Hpx-(tags) locally within the brain of C57BL/6 mice. After inducing an ICH with the autologous whole blood model, Hpx-overexpressing mice were found to have smaller lesion volumes ($p<0.05$) and reduced blood accumulation (red positive pixel count, $p<0.05$) at 72h post-ICH, as identified by quantification of cresyl violet stained brain sections (n=12-13/group). Furthermore, this reduced ICH-induced brain injury was associated with improved neurologic functional recovery as measured by a 24-point neurological deficit scale at 48h and 72h post-ICH ($p<0.05$). To begin identifying the mechanisms involved in Hpx-mediated neuroprotection, histological staining for ferric iron, 4-hydroxynonenal, GFAP, Iba1, heme oxygenase-1, and myeloperoxidase was performed and the distribution of Hpx-tag proteins was evaluated by Western blotting with anti-tag probing of brain homogenates, cerebrospinal fluid, and serum. Hpx-overexpressing mice were found to have significantly increased cortical microgliosis and astrogliosis and peripheral neutrophil infiltration, but no changes in striatal microgliosis and astrogliosis, ferric iron content, heme oxygenase-1 expression, or lipid peroxidation. Additionally, Hpx-tag proteins were found to be highly expressed in all three types of biological specimens evaluated. The increased levels of Hpx in the serum of overexpressing mice were confirmed to be approximately 62% higher by ELISA. These results indicate that overexpression of Hpx in the brain results in the capacity to facilitate the clearance of heme by central and peripheral mechanisms. Specific modulation of local Hpx levels may represent a clinically relevant strategy for the treatment of secondary brain injury following ICH.

Disclosures: **A. Lampert:** None. **J.L. Leclerc:** None. **J. Santiago-Moreno:** None. **A. Dang:** None. **S. Dore:** None.

Poster

324. Stroke Recovery

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 324.30/N23

Topic: C.21.Stroke Recovery

Support: NIH F31 NS086441

NIH R01 NS046400

Title: Haptoglobin improves intracerebral hemorrhage outcomes by modulating angiogenesis

Authors: *J. L. LECLERC[1,2,3], T. ESFANDIARY[1,3], S. DORE[1,2,3,4];
[1]Anesthesiol., [2]Neurosci., [3]Ctr. for Translational Res. in Neurodegenerative Dis., [4]Neurology, Psychiatry, Psychology, and Pharmaceutics, Univ. of Florida, Gainesville, FL

Abstract: Intracerebral hemorrhage (ICH) most often occurs spontaneously and is one of the most devastating stroke subtypes. Following ICH, toxic blood components must be cleared from the brain as part of the tissue repair/scar formation healing process. Angiogenesis is a key physiologic mechanism that facilitates tissue repair following acute injury, but must be tightly regulated to prevent excessive activity and deleterious consequences. After ICH, regulation of angiogenesis within the appropriate range in injured brain regions would allow for delivery of glucose and oxygen to support the energy-requiring reparative processes and facilitate the necessary entry of peripheral cells involved. Haptoglobin (Hp) is an acute phase protein that binds extracorpuscular hemoglobin, thereby directly reducing its oxidative potential, and has also been shown to have potent angiogenic, vasculogenic, and wound healing properties. Using the autologous blood model of ICH, we have shown that Hp overexpression significantly improves anatomical and functional outcomes and reduces oxidative processes. Here, we aimed to confirm our previous results and further characterize the mechanisms by which Hp exerts these neuroprotective effects. Similar to our previous study, Hp was overexpressed in the brain using adeno-associated viral vectors and we used the collagenase-induced spontaneous bleeding model of ICH, which is accompanied by clinically relevant intraventricular hemorrhage. Functional outcomes were assessed by a 24-point neurological deficit score and automated open field locomotor ability, and mice were euthanized at 72h post-hemorrhage to evaluate various ICH outcomes and mechanisms of Hp neuroprotection by histological staining. In line with our previous study, Hp-overexpressing mice demonstrated significantly smaller lesion volumes ($p<0.01$) and less residual blood ($p<0.05$). This reduced ICH-induced brain injury was accompanied by trends towards improved ambulatory ability and less focal neurological deficits at 72h post-hemorrhage ($p<0.07$). Hp-overexpressing mice had significantly reduced PECAM-1 immunoreactivity and tended to have less VEGF immunoreactivity. After correcting for lesion volume, Hp-overexpressing mice retained the significantly reduced PECAM-1 expression, but VEGF expression was increased, collectively suggesting a direct role of Hp in positively modulating angiogenesis after ICH. Hp therapy could represent a new treatment strategy for ICH through a multifactorial mechanism that includes the modulation of important angiogenic processes.

Disclosures: J.L. Leclerc: None. **T. Esfandiary:** None. **S. Dore:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.01/N24

Topic: D.01. Chemical Senses

Support: NIH Grant R01 DC 00997701

NSF Grant DMS-1211952

Title: Asymmetrical transport of food volatiles during retronasal smell

Authors: R. NI[1], M. H. MICHALSKI[3], E. BROWN[3], N. T. T. DOAN[2], J. P. ZINTER, III[2], N. T. OUELLETTE[1], *G. M. SHEPHERD[4];
[1]Dept. of Mechanical Engin. & Materials Sci., [2]Ctr. for Engin. Innovation and Design (CEID), Yale Univ., New Haven, CT; [3]Diagnos. Radiology, [4]Dept. Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The ability of humans to distinguish the delicate differences in flavor between many kinds of cuisines depends mostly on retronasal smell, in which the food volatiles entrained into the airway at the back of the oral cavity are transported in the exhaled air to pass through the nasal cavity and stimulate the olfactory receptor neurons. Nothing is known about the fluid dynamics of this retronasal airflow, particularly how the food volatiles are preferentially carried by retronasal flow toward the nasal cavity rather than by orthonasal flow into the lung. To analyze this mechanism, we obtained CT images of the oronasal airway from a healthy human subject, printed it out using a 3D printer, and analyzed the flow field inside the airway using a particle tracking approach. The results show that, during inhalation, the specific local detailed anatomical structure of the oropharynx creates an air curtain outside a virtual cavity connecting the oropharynx and the back of the mouth, which prevents the food volatiles from being transported into the main stream toward the lung. In contrast, during exhalation, the airflow preferentially sweeps by this virtual cavity and effectively enhances the entrainment of the food volatiles into the main retronasal flow. This asymmetrical transport efficiency is also found to have a nontrivial Reynolds number dependence: it peaks at a range of an intermediate Reynolds number close to 800 because the air curtain effect during inhalation becomes strongest in this range. In summary, this study provides the first experimental evidence to connect the evolutionary geometry of our airway near the oropharynx with the transport efficiency of the food volatiles. It will shed new light on our understanding of retronasal smell and its critical contribution to food flavor.

Disclosures: R. Ni: None. **M.H. Michalski:** None. **E. Brown:** None. **N.T.T. Doan:** None. **J.P. Zinter:** None. **N.T. Ouellette:** None. **G.M. Shepherd:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.02/N25

Topic: D.01. Chemical Senses

Support: FONDECYT 1140520

Title: Chemosensory cilia of olfactory sensory neurons may use glucose from surrounding mucus to satisfy energy demands of odor transduction

Authors: *J. BACIGALUPO[1], P. VILLAR[2], K. BLANCHARD[2], D. VILLALOBOS[2], R. DELGADO[2], C. VERGARA[2], J. G. REYES[3];
[2]Biology, Fac. of Sci., [1]Univ. of Chile, Santiago, Chile; [3]Inst. de Química, P. Univ. Católica de Valparaiso, Valparaiso, Chile

Abstract: About ten olfactory cilia (~60 μm long, 0.2 μm), devoid of inner membranes, project from the apical knob of the single dendrite of an olfactory sensory neuron (OSN). Odorants bind to G-protein coupled receptors triggering the opening of Ca2+-permeable cyclic nucleotide-gated channels (CNGs), mediated by a cAMP-cascade. Ca2+ then activates Cl- channels. Both channels underlie a depolarizing receptor potential. ATP is highly demanded in cilia physiology by a cyclase, ATPases and kinases. The closest mitochondria (2-4) are in the knob. Slow ATP diffusion and limited basal ATP levels suggest the requirement of another ATP source beside mitochondria for sustaining transduction under intense stimulation. Immunohistochemistry revealed glucose transporters in supporting cells (SCs) and ciliary layer of the olfactory epithelium, which contains OSNs cilia and SCs microvilli (Nuñez-Parra et al, PlosOne, 2012). This suggests that glucose could be released to the mucus by SCs and incorporated by the cilia for producing ATP by glycolysis. We confirmed by immunocytochemistry the presence of GLU-3 glucose transporter in OSN cilia and SC microvilli and found that the cilia incorporate a fluorescent glucose analog from the mucus. Glycolytic enzymes were detected by immunoblotting of ciliary membranes. Glycolysis and oxidative phosphorylation inhibitors diminished odor responses in field epithelial and suction pipette recordings. OSNs exhibited fatigue upon removal of glucose (0.2 mM) from extracellular solution. Glucose measurements detected its presence in the epithelial surface. These results suggest that olfactory cilia use two complementary ATP sources, oxidative phosphorylation in the knob and glycolysis in the cilia.

Disclosures: J. Bacigalupo: None. **P. Villar:** None. **K. Blanchard:** None. **D. Villalobos:** None. **R. Delgado:** None. **C. Vergara:** None. **J.G. Reyes:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.03/N26

Topic: D.01. Chemical Senses

Support: NIH Grant 5RO1EB010244

NIH Grant 5DP1CA186693

Title: Epigenetic regulation of olfactory receptor expression during differentiation of single sensory neurons

Authors: *L. TAN, X. S. XIE;
Harvard Univ., Cambridge, MA

Abstract: Mammals sense odors through the massive gene family of olfactory receptors (ORs). Despite the enormous number of OR genes (more than 1,000 in mouse), each olfactory sensory neuron randomly chooses one, and only one, OR for expression. In neurobiology, this "one-neuron-one-receptor" rule has been a longstanding mystery. Recent experiments showed an epigenetic mechanism for maintaining the "one-neuron-one-receptor" rule: Once any ORs are activated during neuronal differentiation, their expression inhibits further OR activation by down-regulating a histone demethylase Kdm1a (also known as Lsd1), an enzyme required for the removal of the repressive histone marker H3K9me3 on OR genes. However, it remains unclear at a quantitative level how a single OR is initiated in the first place. In particular, does a simple scheme of activation and feedback suffice to guarantee the "one-neuron-one-receptor" rule? Here we show theoretically that a simple kinetic model can indeed produce robust OR choices if, and only if, two timescales--slow OR activation by stepwise H3K9me3 demethylation, and fast feedback to turn off Kdm1a--are well separated. In contrast to recent studies, which emphasized the dichotomy between activation and maintenance of OR expression, we suggest that these two phases should not be viewed separately. In fact, it is the ratio between the two timescales that determines to which extent the "one-neuron-one-receptor" rule holds. To achieve the published accuracy (only 2% of neurons express more than one ORs), we predict that OR activation must be as slow as 5-10 days and the feedback as fast as 1-2 hours. Our model further suggests H3K9me3-to-H3K9me2 demethylation as an additional rate-limiting step responsible for the choice of a single OR. We also tested our results via single-cell transcriptomic sequencing of olfactory sensory neurons. Our work provides the theoretical underpinning behind the choice of ORs, and demonstrates how the nervous system utilizes the slow kinetics of epigenetic changes to direct a dramatic outcome neurogenesis. Our conclusions may be generally applicable to other systems where monoallelic expression is desired, and provide guidelines for the design of a synthetic system of singular expression.

Disclosures: L. Tan: None. **X.S. Xie:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.04/N27

Topic: D.01. Chemical Senses

Title: Odor discrimination and detection threshold in mousensor transgenics

Authors: *R. MINA, P. FEINSTEIN, C. D'HULST;
CUNY Grad. Center/Hunter Col., New York, NY

Abstract: Research in understanding the functional properties of odorant receptors that mediate olfaction has been deterred by the difficulty in expressing them in heterologous cells. Our lab has therefore created a reliable method of functionally expressing odorant receptors using transgenic mice, called MouSensors. This enables a dramatic increase of the number of native olfactory sensory neurons expressing a defined odorant receptor. We also designed an *in vivo* imaging protocol to assess the activation of the overexpressed receptors. To determine whether overexpressing a specific odorant receptor changes the odor detection threshold we will perform a proof of principle behavioral experiment using our existing mouse M71 and human OR MouSensors. Genotyped animals will be subjected to a discrimination behavior test using known ligands such as acetophenone and a chosen human odorant. We will compare the threshold of odor detection in drinking water after mice develop an aversion to the odor through lithium chloride injection between transgenes and their wild-type littermates. Preference for odorized water versus non-odorized water will be calculated.

Disclosures: R. Mina: None. **P. Feinstein:** None. **C. D'Hulst:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.05/N28

Topic: D.01. Chemical Senses

Support: DFG CI 222/1-1

NIH grant DC009640

NIH grant DC013576

NIH grant F32DC012004

Title: Comparison of olfactory sensitivity in sensory neurons and behaving animals

Authors: *A. CICHY[1], A. K. DEWAN[1], J. ZHANG[1], D. RINBERG[2], T. BOZZA[1];
[1]Neurobio., Northwestern Univ., Evanston, IL; [2]Dept. of Neurosci. & Physiol., NYU Neurosci. Inst., New York, NY

Abstract: The ability to detect and discriminate a broad range of environmental chemicals and to translate that information into meaningful cellular responses is essential for the survival of all

individuals. This challenge is met by different chemosensory systems. Among those, the main olfactory system is indispensable for the perception of volatile odorants. While the peripheral odor detection mechanisms have been extensively investigated, little is known about how sensitivity of individual olfactory sensory neurons (OSNs) relates to the detection threshold in behaving animals. Furthermore, there has been a long-standing disparity between the sensitivity of physiological responses of olfactory neurons and behavioral thresholds measured in intact animals. Using a combined approach of gene targeting, electrophysiology, *in vivo* imaging, and behavior, we examined odor detection thresholds of single OSNs in the olfactory epithelium, glomeruli in the olfactory bulb, and behaving mice. We focused on a class of main olfactory receptors - the Trace Amine-Associated Receptors (TAARs). Our results indicate that TAARs contribute significantly to setting behavioral thresholds to specific odors (amines). Perforated patch-clamp recordings from knobs of TAAR-expressing OSNs and *in vivo* imaging of the corresponding glomeruli in awake mice exhibited similarly low detection thresholds. In comparison, *in vivo* imaging of the same glomeruli in anesthetized mice showed a pronounced decrease in sensitivity. Behavioral detection thresholds reported by a go-no go assay were slightly higher than those observed in glomerular responses of awake animals. Together, our results show that behavioral thresholds can closely follow the sensitivity of one or a few populations of genetically defined OSNs. In addition, active processes in awake animals, such as sniffing, make an important contribution to odor sensitivity. More generally, our combinatorial approach allows us to characterize for the first time how chemical detection at the level of OSNs relates to olfactory perception in the behaving animal.

Disclosures: **A. Cichy:** None. **A.K. Dewan:** None. **J. Zhang:** None. **D. Rinberg:** None. **T. Bozza:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.07/N29

Topic: D.01. Chemical Senses

Support: NIH Grant 5RO1DC013087

Title: Switching of pup-directed behaviors by vomeronasal receptor inputs

Authors: *Y. ISOGAI[1], H.-Y. AHN[1], M. I. LOVE[2], Z. WU[1], V. HUA[1], D. BAMBAH-MUKKU[1], R. IRIZARRY[2], C. DULAC[3];
[1]Harvard Univ., Cambridge, MA; [2]Dana Farber Cancer Institute, Harvard Med. Sch., Boston, MA; [3]HHMI, Harvard Univ., Cambridge, MA

Abstract: One of the fundamental problems in neuroscience is to elucidate the neural circuits underlying the sociality of animals. Especially in mice, which heavily rely on chemosensory signals to guide their behaviors, the vomeronasal organ (VNO) plays a vital role in social and defensive behaviors. The vomeronasal circuits therefore serve as an attractive entry point to study social behavior circuits in detail. Our previous study linked approximately 90 vomeronasal receptors to socially and physiologically relevant stimuli. This study led us to conclude that VRs encode highly specific identity (e.g., gender, species) or physiologically relevant information (e.g., stress and reproductive information). We therefore hypothesized that the VNO functions similarly to a switchboard, by which individual receptors signal specific information to the brain, critical for animals' behavioral decisions. To test this hypothesis, we investigated pup-directed behaviors, in which the VNO has been previously demonstrated to play a critical role. Importantly, wild type virgin males display pup-directed aggression whereas males lacking the VNO function, TrpC2 knockout mice for example, strikingly display parental behaviors. We screened specific vomeronasal receptors activated by pups by a combination of RNA-seq and high-throughput RNA *in situ* hybridization and successfully identified specific vomeronasal receptors tuned to pup-associated cues. We found that male mice lacking a subset of these receptors displayed dramatically reduced pup-directed aggression and increased parental behaviors. Taken together, these specific VNO "switches" controlling pup-directed behaviors will serve as a powerful platform to dissect the neural circuits underlying one of the most important aspects of social life – nurturing the offspring.

Disclosures: **Y. Isogai:** None. **H. Ahn:** None. **M.I. Love:** None. **Z. Wu:** None. **V. Hua:** None. **D. Bambah-Mukku:** None. **R. Irizarry:** None. **C. Dulac:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.08/N30

Topic: D.01. Chemical Senses

Support: NIH Grant DC005964

Title: Functional clustering of mouse vomeronasal sensory neurons through exhaustive calcium imaging

Authors: *D. LEE, T. E. HOLY;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Examining neuronal activity can provide insight into a neuron's molecular features and upstream/downstream neural circuits. Here, we recorded neuronal activity from approximately 10,000 highly heterogeneous sensory neurons in the mouse vomeronasal organ,

and categorized them by comparing their ligand-evoked responses. To examine neuronal activity from such a large number of pheromone-sensing neurons, we imaged transgenic mice expressing a genetically encoded calcium indicator (GCaMP3) with a variant of light sheet microscopy called objective-coupled planar illumination (OCPI) microscopy. Among the entire population recorded, we performed an in-depth analysis on ~1500 neurons responsive to previously known ligands. Neurons showing similar responsiveness across the entire panel of ligands, all presented at 10 μM, were categorized into the same cluster, resulting in 30 different clusters. By examining responses at multiple ligands concentrations, it was possible to further subdivide the neurons based on their sensitivity. The existence of distinct subtypes - rather than a continuum of sensitivities - is consistent with the presence of two or more receptor genes with very similar tuning profiles but differing in terms of sensitivity. We asked whether these types and sub-types might correspond to distinct receptor genes by analyzing two other properties of these neurons. First, because spontaneous activity has been shown, in other olfactory systems, to depend upon the specific receptor gene, we measured the standard deviation of fluorescence intensity in the absence of ligands. The mean standard deviation of the neurons in each cluster varied among clusters, suggesting that sensory neurons in different clusters exhibit distinct levels of spontaneous activity. Second, we measured the duration of neuronal response upon ligand treatment. Interestingly, neurons in the same cluster exhibited dichotomous response durations to the same ligands, but subclusters exhibited temporally-uniform responses. This indicates that response duration could further subdivide the functional clusters defined by ligand selectivity. Together, our exhaustive calcium imaging and analysis of ligand selectivity, sensitivity, spontaneous activity, and response duration provided functional categorization of heterogeneous neurons to a resolution consistent with single receptor genes.

Disclosures: D. Lee: None. **T.E. Holy:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.09/N31

Topic: D.01. Chemical Senses

Title: Identification of new ligands and analysis of the signal processing in the mouse olfactory Grueneberg ganglion

Authors: *F. MOINE, J. BRECHBÜHL, V. FRANZEN, M. NENNIGER TOSATO, M.-C. BROILLET;
Dept. of Pharmacol. and Toxicology, Univ. of Lausanne, Lausanne, Switzerland

Abstract: Organisms take advantage of their olfactory system to detect and analyze the odorants and pheromones present in their chemical environment. The mouse olfactory system is

composed of olfactory subsystems which are the main olfactory epithelium, the vomeronasal organ, the septal organ and the Grueneberg ganglion (GG). We have previously shown that, in rodents, the GG mediates the detection of volatile cues encoding for impending danger, such as alarm pheromones and predator-derived kairomones. The kairomones are molecules emitted by mice predators and implicated in interspecies danger communication. Several kairomones share a similar chemical signature with the mouse alarm pheromone (AP) that we characterized by SPME/GC-MS, the 2-sec-butyl-thiazoline (SBT). They possess a heterocyclic sulfur- or nitrogen-containing motif. The SBT alarm pheromone is indeed chemically related to kairomones emitted by mice predators, such as the 2,4,5-trimethylthiazoline (TMT) from the red fox or the 2-propylthietane (2-PT) from the stoat. We have shown that these chemical danger cues induce specifically calcium transients in mouse GG neurons. We are now tracking the circuitry triggered by SBT after this activation of mouse GG neurons, using the expression of immediate-early genes. These experiments are performed in control versus GG-axotomized mice focusing on the brain areas involved in fear detection. In parallel experiments, we have also detected by HS-SPME/GC-MS new GG ligands among which new families of chemical molecules emitted by mice predators. We then verified the activation of the GG neurons generated by these new ligands via calcium imaging experiments and their fear-inducing properties in mice by behavioral analysis. We could thus determine further the molecular receptive range of mouse GG neurons. Moreover, immunohistochemical and molecular techniques help us identify multiple signaling elements present in mouse GG neurons. In summary, these results confirm that the Grueneberg ganglion plays a dual role in detecting both intra- and interspecific chemical molecules. This study is supported by a Swiss National Foundation grant (SNF 3100A0-125192).

Disclosures: **F. Moine:** None. **J. Brechbühl:** None. **V. Franzen:** None. **M. Nenniger Tosato:** None. **M. Broillet:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.10/N32

Topic: D.01. Chemical Senses

Support: NIH grant R01 DC011286

NIH grant R01 MH067528

NIH grant P30 NS052519

Title: Spatial patterns of receptor neuron input to the olfactory bulb are correlated with odor-evoked fMRI maps of glomerular activity

Authors: *B. G. SANGANAHALLI[1], M. R. REBELLO[2], P. HERMAN[1], G. M. SHEPHERD[3], J. V. VERHAGEN[2], F. HYDER[1];
[1]Diagnos. Radiology, [2]The John B. Pierce Lab., [3]Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Functional imaging signals arise from distinct metabolic and hemodynamic events at the neuropil, but how these processes are influenced by pre- and post-synaptic activities is needed for quantitative interpretation of stimulus-evoked mapping data. The olfactory bulb (OB) glomeruli, spherical neuropil regions with well-defined neuronal circuitry, can provide insights into this issue. Optical calcium-sensitive fluorescent dye imaging (OICa2+) reflects dynamics of pre-synaptic input to glomeruli, whereas high-resolution functional magnetic resonance imaging (fMRI) using deoxyhemoglobin contrast reveals neuropil function within the glomerular layer where both pre- and post-synaptic activities contribute. We imaged odor-specific activity patterns of the dorsal OB in the same anesthetized rats with fMRI and OICa2+ and then co-registered the respective maps to compare patterns in the same space. Maps by each modality were highly reproducible as trial-to-trial patterns for a given odor, overlapping by ~80%. Maps evoked by ethyl butyrate and methyl valerate for a given modality overlapped by ~80%, suggesting activation of similar dorsal glomerular networks by these odors. Comparison of maps generated by both methods for a given odor showed ~70% overlap, indicating similar odor-specific maps by each method. These results suggest that odor-specific glomerular patterns by high-resolution fMRI primarily tracks pre-synaptic input to the OB. Thus combining OICa2+ and fMRI lays the framework for studies of OB processing over a range of spatiotemporal scales, where OICa2+ can feature the fast dynamics of dorsal glomerular clusters and fMRI can map the entire glomerular sheet in the OB.

Disclosures: B.G. Sanganahalli: None. **M.R. Rebello:** None. **P. Herman:** None. **G.M. Shepherd:** None. **J.V. Verhagen:** None. **F. Hyder:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.11/N33

Topic: D.01. Chemical Senses

Title: Use of the medicinal chemistry rules in olfactory detection as a new point of view in odorant classification

Authors: *E. POIVET, N. TAHIROVA, L. XU, S. FIRESTEIN;
Biol. sciences, Columbia Univ., New York, NY

Abstract: Since the first identification of mammalian olfactory receptors (ORs) in 1991, the deorphanization of the ORs appears to be laborious. Each olfactory sensory neuron (OSN) expresses a single OR, and all axons from OSNs expressing that particular OR project to the same glomeruli in the olfactory bulbs, suggesting an “odor-map” in the brain. While some ORs can accept multiple chemically distinct odorants, others may be more specific. Furthermore, two different ORs can have overlapping panels of odorant ligands. These properties make the “odor-map” complicated. In addition, the classification system of odorants offered by organic chemistry does not always recapitulate a biologically relevant function. Associating an odorant's chemical structure to its perception is a long-standing challenge. Since medicinal chemistry emphasizes biological function over chemical form, we are testing whether odorants related by medicinal chemistry rules are treated by ORs as being similar, using calcium imaging on mouse dissociated OSNs. Starting from the acetophenone, we have built a panel of six odorants using medicinal chemistry rules to test the hypothesis that, among odorants, heteroaromatic rings can substitute for benzene rings, with ORs exhibiting a predictable preference between them. The ORs response patterns to our odorants panel lead to an odorant classification totally different to the one expected if based on organic chemistry. We then applied our panel of six odorants on mice in a habituation-dishabituation behavior test. The results from this behavioral test confirmed our observations in calcium imaging in the dissociated OSNs. Together, our results suggest that electronegativity inside the ring is more important for odorant specification than the nature of the ring itself, and that the organic chemistry classification of molecules may not be relevant when applied to olfaction.

Disclosures: **E. Poivet:** None. **N. Tahirova:** None. **L. Xu:** None. **S. Firestein:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.12/N34

Topic: D.01. Chemical Senses

Support: Office of Naval Research grant (Grant#: N00014-12-1-0089)

Title: Role of sensory neurons in intensity dependent behavioral response switch in *Drosophila*

Authors: H. RONG[1], P. DAS[3], A. LUBE[3], Y. BEN-SHAHAR[2], *B. RAMAN[3];
[1]Biomed. Engin., [2]Biol., Washington Univ., St. Louis, MO; [3]Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: To survive and propagate, animals must be able to decode complex chemosensory signals and respond with appropriate behaviors. Thus, many animals evolved highly sensitive, dedicated chemosensory systems that are finely tuned to the qualitative and quantitative aspects

of their chemical environments. The molecular identification of diverse chemosensory receptors in different animal species has greatly advanced our understanding of how the nervous system perceives and decodes odor identities. However, how the nervous system decodes odor intensities remains poorly understood, especially considering that some odors can elicit contrasting behavioral responses (e.g., attraction versus repulsion) at different odor intensities. Consequently, to address this fundamental question in sensory neurobiology we propose to take advantage of the relatively simple, and well-studied olfactory system of the fruit fly *Drosophila* melanogaster. Using T-maze assay, we found that some odorants (not all) that were highly attractive to flies at low concentrations, became repulsive above an odor-specific intensity threshold. Preliminary data revealed that when the intensity of such odorants increases, the activity of sensory neurons that were highly sensitive to those odorants switched from a regular spiking (< 100 Hz) to a high firing bursting regime (>>100 Hz). This transition in low-threshold neurons was correlated with the recruitment of neural activity of independent high-threshold olfactory neurons that were not sensitive to that odorant. While neither class of neurons by themselves were sufficient to fully explain the behavioral response preference and its switch, we found that a linear model integrating the information from the two response types was sufficient to explain the fly behavior in the T-maze assay to small repertoire of odorants presented at varying intensities. In sum, our results indicates that the fly's olfactory system may employ a simple and efficient mechanism to encode odor intensity by integrating information from a relatively small subset of olfactory response types.

Disclosures: H. Rong: None. **P. Das:** None. **A. Lube:** None. **Y. Ben-Shahar:** None. **B. Raman:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.13/N35

Topic: D.01. Chemical Senses

Support: NIH Grant R01 DC012943

Title: Differential effects of adaptation on odor discrimination

Authors: *S. HANEY[1], D. SAHA[2], B. RAMAN[2], M. BAZHENOV[1];
[1]Univ. of California, Riverside, Los Angeles, CA; [2]Dept. of Biomed. Engin., Washington Univ., St. Louis, MO

Abstract: Animals are exposed to a complex and dynamic olfactory environment. To represent and respond to this environment, the olfactory system utilizes combination of spatial (across population of neurons) and temporal (rate of firing and/or synchrony of firing) coding strategies.

If, however, the olfactory stimulus changes over time, as it commonly does in the natural environment, the ability of the olfactory system to use time as a coding variable can be jeopardized. In many other sensory systems, including vision and audition, adaptation plays a critical role in resetting neural systems to its baseline state to allow for a robust representation of temporally structured stimuli. We show this strategy is also employed successfully in the insect olfactory system. Responses of the olfactory receptor neurons (ORNs), the primary sensory neurons in olfaction, adapt with odor-dependent properties that adds complexity to the temporal component of odor encoding. We quantify the spectrum of the ORN adaptation *in vivo* and use these data to construct a detailed kinetic model in silico. We then use our network model, including populations of ORNs and antennal lobe projections neurons and local inhibitory interneurons, to investigate the role of the ORN adaptation in coding. Particularly we asked the question how adaptation affects reliability of the odor representation when several odors are presented simultaneously, mimicking a complex olfactory environment. We found that strong adaptation allows for a foreground odor to be represented faithfully even in the presence of another different background odor. When background and foreground odors were very similar, however, the adaptation increased the likelihood of misclassification. We employed, these findings to propose a novel behavioral experiment where adaptation can be exploited to produce 'olfactory illusions' that may result in misclassification of odorants. Our results provide a novel insight into the strengths and vulnerabilities of a ubiquitous coding strategy, adaptation, in the specific case of insect olfaction.

Disclosures: **S. Haney:** None. **D. Saha:** None. **B. Raman:** None. **M. Bazhenov:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.14/N36

Topic: D.01. Chemical Senses

Support: McDonnell Center for Systems Neuroscience

Office of Naval Research (Grant#: N00014-12-1-0089)

Title: A functional role for off-transients in olfactory coding

Authors: ***D. SAHA**, C. LI, W. PADOVANO, B. RAMAN;
Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Sensory stimuli often evoke temporal patterns of spiking activity across a population of neurons at each level of the neural processing hierarchy. A fundamental problem in sensory neuroscience is determining what stimulus-specific information is encoded during distinct dynamical phases of ensemble neural activity and determines the behavioral relevance of the

information that is encoded in these epochs. In the insect olfactory system, odorants are detected by olfactory receptor neurons (ORNs) in the antenna, which transduce chemical stimuli into trains of action potentials. The ORN signals are relayed downstream to the antennal lobe, where spatiotemporal patterns of activity across ensembles of projection neurons represent odors. The odor-evoked projection neuron responses are elaborate and change most rapidly after stimulus onset and offset (referred to as on-transient and off-transient responses, respectively). For lengthy odor presentations, the antennal lobe activity converges to a steady state within ~1.5 s of stimulus onset. These dynamic neural activity patterns are repeatable across trials and contain information about odor identity and intensity. In this study, we examined what information is encoded in the neural activity that follows stimulus termination. We found that these OFF responses also contain information about stimulus identity and intensity. We reveal a striking relationship between the ON and OFF responses and an important behavioral role for OFF responses. Finally, we discuss how the signal processing and representation approach our data suggests may be a conserved approach to actively indicate the absence of a stimulus.

Disclosures: **D. Saha:** None. **C. Li:** None. **W. Padovano:** None. **B. Raman:** None.

Poster

325. Olfactory Receptors and Sensory Detection

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 325.15/N37

Topic: D.01. Chemical Senses

Support: ONR Grant N00014-12-1-0089

Children's Discovery Institute Grant MD-II-2014-411

Title: Spontaneous firing of sensory neurons modulates the gain in the downstream circuit of a simple olfactory system

Authors: ***N. KATTA**, M. O'NEILL, D. SAHA, B. RAMAN;
Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: In locusts and other insects, odorants are transduced into electrical signal by the olfactory receptor neurons and transmitted to central circuits for further processing. The initial encounter with an odorant can occur under diverse ambient conditions. For example, environmental variables important to chemical sensing such as flow rate, temperature, humidity etc. can change independently or in combination with one another, yet the odor detection and recognition has to remain invariant. Previous studies have shown that these exogenous variables do influence the responses of the sensory (first-order) neurons and therefore modulate the central circuits. However, how the sensory neuron activity is manipulated to engage or disengage adaptive gain control mechanisms in the following circuits is yet to be understood. It is possible

that the magnitude of the stimulus-evoked response in the receptor neurons, their spontaneous activity, or a combination of these factors can change how information about a chemical cue is processed downstream. To this end, we studied the effects of ambient conditions on olfactory sensing and downstream processing by modulating flow rate of odorant delivery and relative humidity. We examined activity at four levels of the olfactory system: individual olfactory receptor neurons, population receptor neuron activity as assayed by electroantennograms, individual projection neurons in the antennal lobe of the brain, and population antennal lobe activity as assayed by local field potential recordings obtained from the mushroom body. Our results reveal that flow rate modulation primarily changed the overall response magnitudes evoked by different odorants without altering the baseline sensory neuron activity. These changes in sensory input appeared to have had little effect on the downstream neural activity. Increases in the relative humidity, however, caused a decrease in both response magnitude and spontaneous activity of the receptor neurons, which brought about a significant compensatory change in the spontaneous and odor-evoked activity of the second-order neurons in the antennal lobe. Further, our preliminary data suggests that these manipulations did not induce any significant change in response time constants or other finer temporal features of processing in these early circuits. Taken together, our data suggests that these changes in the downstream circuit of the brain were primarily due to changes in spontaneous baseline activity of the first-order neurons.

Disclosures: **N. Katta:** None. **M. O'Neill:** None. **D. Saha:** None. **B. Raman:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.01/N38

Topic: D.01. Chemical Senses

Support: NIH Grant R01DK102918

Title: Food deprivation inhibits mitral cell firing in the mouse main olfactory bulb

Authors: ***K. O'CONNELL**, J. W. GAMMONS, W. WEI;
Physiol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: There is increasing evidence from both rodent and human behavioral studies that nutritional status and body weight influence olfactory behaviors. Since the main olfactory bulb (MOB) receives extensive inputs from other brain regions via centrifugal fibers, it is likely that these inputs contribute significantly to the modulation of olfactory processing in response to changes in an organism's metabolic state. However, presently there is a significant gap in our understanding of how these centrifugal inputs influence olfactory processing and behavior as a

function of the intrinsic homeostatic needs of the animal. It is well established that CNS control of body weight homeostasis and energy balance occurs initially in the hypothalamus, which subsequently projects to a plethora of other brain regions, including the olfactory bulb. In this study, we found that overnight food deprivation is associated with a ~6-fold decrease in the spontaneous action potential firing rate of mitral/tufted (M/T) cells compared to ad libitum-fed controls (Ad lib: 22.8 ± 7.1 Hz; Fast: 3.6 ± 1.0 Hz, $p = 0.01$). This change in M/T cell firing rate was also associated in a change in the rheobase of M/T cells. In the presence of synaptic blockers, more somatic current injection was required to evoke an AP in M/T cells from fasted mice compared to that required for neurons from ad libitum-fed animals. This suggests that food deprivation alters the intrinsic excitability of M/T cells, possibly by modulating ion channel expression in response to nutrient deprivation. However, we also found evidence that food deprivation alters inhibitory synaptic input to M/T cells. We observed an increase in the immediate-early gene cFos in the granule cell (GC) layer of the MOB following overnight fasting in mice. Since GC cells are key GABAergic interneurons that shape M/T cell output and are primary postsynaptic targets of centrifugal fiber (CFF) inputs from the rest of the brain (including the hypothalamus), CFF activation of GCs may contribute to the decrease in M/T cell firing via increased inhibitory GABAergic or peptidergic input to M/T cells. Consistent with this, we observed a change in the IPSC frequency in M/T cells from fasted mice compared to ad libitum fed animals. Taken together, these results suggest that hypothalamic signals of metabolic status may influence the activity of M/T cells and thus, olfactory function, with implications for food preference and palatability.

Disclosures: **K. O'Connell:** None. **J.W. Gammons:** None. **W. Wei:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.02/N39

Topic: D.01. Chemical Senses

Support: IFR NeuroSud

Title: Odor processing dynamics in the olfactory system: what is the impact of obesity?

Authors: ***C. MARTIN**[1,2], Y. CHELMINSKI[2], H. GURDEN[2], N. MEUNIER[3];
[1]CNRS, Orsay, France; [2]IMNC, Univ. Paris-Sud, Orsay, France; [3]INRA Univ. Versailles St-Quentin, Jouy-en-Josas, France

Abstract: Over the past three decades, obesity rates have expanded and have become a serious public health issue. This pathology is defined as the condition in which an excess in body fat is harmful for health, and characterized by a deregulation of food intake. The bidirectional

relationship between the olfactory system and food intake regulation and motivation for food is still unclear and needs further insights. The objective of this study is to test whether the nutritional status modulates odor representations at the first levels of odor processing. Our model is a strain of genetically obese mice (ob/ob mice), deficient in leptin hormone. We investigated odor-evoked electrical response of the olfactory epithelium recorded by electro-olfactogram (EOG) and oscillations of the local field potential in the main olfactory bulb in both obese and control mice. Mice were challenged for an operant test of odor discrimination in a go/no-go task and odor evoked responses compared between trained and untrained animals. In this context, ob/ob mice did not show deficits for odor learning. However, examining the EOG characteristics and beta (15-40 Hz) and gamma (60-100 Hz) frequency oscillations characteristics of odor processing and plasticity, we found a reorganization of electrical activities in the olfactory bulb and epithelium.

Disclosures: **C. Martin:** None. **Y. Chelminski:** None. **H. Gurden:** None. **N. Meunier:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.03/N40

Topic: D.01. Chemical Senses

Support: DC006213

DC011554

Title: Optogenetic activation of olfactory sensory neurons in the nose drives rhythmic activity in widespread brain areas

Authors: ***A. H. MOBERLY**, M. MA;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Respiration is not only influenced by autonomic demands, but also by emotional states (fear, anxiety, anger, etc.). On the other hand, conscious control of respiration rate (in the form of rhythmic, nasal breathing) can affect physiology, mood, and cognition. In rodents, nasal respiration entrains rhythmic neural activity in many brain regions including olfactory centers (olfactory bulb, olfactory tubercle, and pirifom cortex) somatosensory cortex, and limbic structures (amygdala and hippocampus). Thus, respiratory input is well positioned to serve as a reference for the synchronization of brain activity across widespread regions. However, the source of respiration-locked neural activity remains unclear. Because these low-frequency rhythms are decoupled from the breathing cycle when inspired air bypasses the olfactory epithelium, primary olfactory sensory neurons (OSNs) may be the source of respiration-locked input to the brain. Since OSNs not only respond to odorants but also to mechanical stimulation

they may provide an early representation of airflow that propagates to olfactory and other brain regions. We confirmed that OSN activation can influence widespread field potential oscillations by stimulating OSNs in the nasal epithelium of OMP-ChR2 mice in combination with multisite local field potential (LFP) recordings. Light stimulation evoked strong oscillatory activity at stimulation frequencies higher than the animal's baseline respiration rate. We observed this result in the barrel cortex and hippocampus - two areas recently discovered to express respiratory-locked rhythms. These results suggest that peripheral information provided by OSNs is able to drive widespread neural activity.

Disclosures: A.H. Moberly: None. **M. Ma:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.04/N41

Topic: D.01. Chemical Senses

Support: NIH R00 DC011780

NIDA T32 DA07290

Title: Plasticity in Arc-transcribing accessory olfactory bulb internal granule cells following intermale aggression

Authors: *H. L. CANSLER, J. P. MEEKS;
UT Southwestern, Dallas, TX

Abstract: The mechanisms underlying the brain's ability to rapidly process sensory information and produce appropriate behaviors is an area of much recent interest. The accessory olfactory system (AOS) is well suited for approaching these questions because it is a relatively simple and compact system that regulates innate, sex-typical behaviors, such as reproductive behavior and territorial aggression. The accessory olfactory bulb (AOB) is the first neural circuit to process chemosignals from the sensory periphery, making it a critical link between sensation and downstream circuits known to guide behavior. Despite decades of research, specific plastic changes that occur in the AOB in response to salient behavioral events remain poorly understood on a cellular and molecular level. We investigated the AOB circuit response to intermale territorial aggression using the immediate early gene Arc, which indicates recent activity and is implicated in diverse forms of plasticity throughout the brain. We found that adult resident C57BL6/J males significantly upregulated Arc protein expression in posterior internal granule cells (IGCs) compared to controls following a 10-minute exposure to a novel Balb/cJ male intruder. Arc protein expression peaks 1-2 hours after behavior and returns to baseline by 4 hours. Exposure to soiled bedding is sufficient to produce this effect, and vomeronasal input is

required, as indicated by experiments with Trpc2-/- mice. We have also found that the Arc gene is necessary for the normal expression of aggressive behavior in the resident-intruder paradigm. One week of solo housing is required for robust aggression from residents, and one-day residents as well as Arc-/- one-week residents exhibit significantly decreased aggressive behavior. This finding implicates Arc in an AOS-mediated behavior for the first time. We used transgenic mice that express GFP in Arc-transcribing cells (Arc-d4EGFP-BAC mice) to investigate the physiological effects of Arc transcription. We targeted patch-clamp recordings to GFP+ and GFP- IGCs during the 4-8 hours after behavior. We found that Arc-expressing IGCs exhibit a fast-spiking phenotype when depolarized with a current injection. The same group also exhibits decreased Ih depolarization when hyperpolarized with a current injection. This suggests that these IGCs either undergo a plastic change following the resident-intruder paradigm, or that Arc expression is specific to a subpopulation of IGCs with these intrinsic properties. These data represent a step towards understanding sensory-mediated plasticity in circuits that guide innate, sex-typical behaviors.

Disclosures: **H.L. Cansler:** None. **J.P. Meeks:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.05/N42

Topic: D.01. Chemical Senses

Support: Grant-in-Aid for Scientific Research

Title: Synaptic plasticity in the olfactory bulb underlying aversive olfactory learning is inhibited by ER stress

Authors: *F. OKUTANI[1], J. TONG[2], H. KABA[2];
[1]Kochi Med. Sch,, Kochi, Japan; [2]Physiol., Kochi Med. Sch., Nankoku, Japan

Abstract: The endoplasmic reticulum (ER) is an organelle in which secretory and transmembrane proteins are folded or processed, and is susceptible to various irritants, such as tunicamycin (TM), that provoke the accumulation of unfolded protein response and induce ER stress in the lumen, which is linked to neuronal death in various neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and many others. It is well known that patients suffer from olfactory dysfunction in these diseases. Therefore we examined the inhibitory effect of ER stress on synaptic plasticity in the rat olfactory bulb (OB) as a model of aversive olfactory learning. Before eye opening young rats depend on somatosensory and olfactory function for survival, as they can learn their dam's odor and approach her without visual information. They do this in part by learning their mother's odor as a conditioned stimulus

that is paired with an unconditioned somatosensory stimulus given by maternal care. We have shown that synaptic plasticity in the OB underlies aversive olfactory learning. In order to establish aversive olfactory learning, pairing of an artificial odor and foot-shock during training on postnatal day (PND) 11 is required. Rats show aversion to the odor on the next day. Infusion of TM into the OB during odor exposure on PND 11 impaired olfactory learning tested on PND 12 in a dose-dependent manner. Short-term memory, however, was maintained even after TM infusion because rats show aversion to the odor at the testing one hour after the training. Electrophysiology using OB slices of PND 11 rats enables us to observe long-term potentiation (LTP) induced in field excitatory postsynaptic potentials slope in the granule cell layer, which is evoked by antidromic stimulation of the lateral olfactory tract. TM administration has an inhibitory effect on the late phase of LTP without affecting the early phase of LTP. These results are consistent each other to suggest that ER stress impaired aversive olfactory learning by inhibiting synaptic plasticity in the OB.

Disclosures: F. Okutani: None. **J. Tong:** None. **H. Kaba:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.06/N43

Topic: D.01. Chemical Senses

Title: Impact of olfactory fear conditioning on olfactory tuning in cortical and medial amygdaloid nuclei

Authors: V. SAVAGE, G. MINOGUE, T. REDMOND, A. ROCHA-CARTAGENA, *G. A. COUSENS;
Drew Univ., Madison, NJ

Abstract: Remarkable progress has been made in recent years in characterizing features of odor representation in piriform cortex. Less is known about odor representation in other primary olfactory regions, including cortical and medial amygdaloid nuclei. Here we show that neurons within cortical and medial amygdaloid nuclei of urethane-anesthetized rats exhibited odor-selective alterations in firing rate often in phase with the ongoing respiratory cycle. Cells exhibited a range of tuning breadths to molecularly distinct odorants, and adjacent cells were often similarly tuned. Preliminary data suggests that prior olfactory fear conditioning enhanced recruitment of cells responsive to the conditioned odor. Current work is examining regional differences odor responsivity and selectivity across amygdaloid nuclei with differing patterns of innervation by main and accessory olfactory bulbs. These findings suggest that cortical and medial amygdaloid nuclei contribute to odor representation and that prior learning can alter olfactory tuning.

Disclosures: **V. Savage:** None. **G. Minogue:** None. **T. Redmond:** None. **A. Rocha-Cartagena:** None. **G.A. Cousens:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.07/N44

Topic: D.01. Chemical Senses

Title: Dynamics of activity in main olfactory bulb granule cells during associative odor learning

Authors: ***B. N. CAZAKOFF**, S. D. SHEA;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Odors are critical to the survival and reproductive success of many organisms. In order to guide appropriate behaviour, the brain must not only form accurate representations of these odor identities, but also flexibly encode their association with positive or negative outcomes. Several brain regions display activity that reflects odor valence, including the main olfactory bulb (MOB). For example, mitral/tufted cells (MTs), which receive input from peripheral receptor neurons and constitute MOB output, exhibit differences in their activity depending on whether or not the odor is rewarded (Kay and Laurent 1999, Doucette and Restrepo 2008). How such divergent activity arises is not known, but several lines of evidence implicate intrinsic MOB inhibitory neurons known as granule cells (GCs). GCs are the primary target of cortical and other centrifugal feedback to the MOB, and they make reciprocal dendrodendritic synapses that inhibit MTs. Therefore, they are well placed to modulate MT activity according to changes in stimulus value. Recent technical advances have enabled examination of GC activity in awake animals using neurophysiology and imaging methods (Cazakoff et al 2014, Kato et al 2013). Nevertheless, whether GC activity reflects odor valence is unknown. We are using 'loose patch' electrophysiology to monitor GC spiking activity in awake, head-fixed mice actively learning to associate odors with positive (delivery of sucrose solution) or negative (delivery of quinine solution) outcomes. We first trained mice to reliably lick for sucrose reward upon detection of any of a set of 8 odors. Subsequently, when an arbitrarily chosen odor becomes associated with delivery of an aversive quinine solution, the mice learn to withhold licking to that odor to avoid the tastant. Consistently, mice rapidly achieve reliable and accurate performance of this behavior. Following the training period they are able to quickly (within ~20-60 trials) form associations of novel odors with sucrose and quinine and can also learn reversals of a previous association. Having established this rapid odor association behavior, we are now recording from MOB neurons, including GCs, during ongoing learning of this task. These results will provide important insight into the role of GCs in olfactory processing and the greater role of inhibition in shaping sensory responses in context.

Disclosures: **B.N. Cazakoff:** None. **S.D. Shea:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.08/N45

Topic: D.01. Chemical Senses

Support: NSERC discovery grant (418451-2013)

Title: Noradrenergic blockage in the olfactory bulb during similar odor discrimination learning leads to impaired odor discrimination and pattern separation in rats

Authors: ***A. M. SHAKHAWAT**, A. GHEIDI, I. T. K. MACINTYRE, Q. YUAN;
Fac. of Med., Mem. Univ., St John's, NL, Canada

Abstract: Norepinephrine (NE) is critical for olfactory learning and odor discrimination in both neonatal and adult rodents. In adult rats and mice, blockage of the olfactory bulb adrenoreceptors impairs fine odor discrimination and reduces synchronized firing of mitral cells in the olfactory bulb in response to the rewarded odor. How NE manipulation in the olfactory bulb influences the odor coding in cortical structures such as the piriform cortex is not well understood. Here, using cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) of an immediate early gene Arc, we studied how blockage of adrenoceptors in the olfactory bulb during odor discrimination training affects odor representations in the piriform cortex. CatFISH enables visualization of Arc at two time points in the same animals. Two ensembles activated by the same odor (rewarded) were used to index the stability of Arc activation, while two ensembles activated by two different odors (rewarded and unrewarded) were used to study pattern separation in the piriform cortex. Consistent with previous reports, local bulbar infusion of both α and β-adrenoreceptors antagonists (α-receptor antagonist Phentolamine, 10 mM and β-receptor antagonist Alprenolol, 120 mM) impaired difficult similar odor discrimination learning. Rats given these antagonists needed significantly more trials than the saline infused group to reach the same level of successful odor discrimination. The neural representations of the rewarded odor in the anterior piriform cortex, revealed by the visualization of Arc+ cells, were similar in the two groups when learning took place. The number of Arc+ cells and the reliability of activated cells when the animals were exposed to the same odor twice during the catFISH procedure, were not different in the two groups. However, at an earlier time point when the saline infused group learned but the drug group did not, we observed an increase in the reliability of activated neural ensembles to the rewarded odor in the saline group compared to the drug group. Furthermore, successful odor discrimination learning also promoted ensemble separation in the piriform cortex when both rewarded and unrewarded odors were tested.

Together, NE blockage in the olfactory bulb impaired similar odor discrimination by reducing the stability of pyramidal cell activation and pattern separation in the piriform cortex.

Disclosures: **A.M. Shakhawat:** None. **A. Gheidi:** None. **I.T.K. MacIntyre:** None. **Q. Yuan:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.09/N46

Topic: D.01. Chemical Senses

Support: DC003906 from the NIDCD to D.A.W

Title: Transient asymmetry in primary and higher order olfactory cortex during odor learning

Authors: ***Y. COHEN**[1], D. PUTRINO[2], D. A. WILSON[1];
[1]EBI, Nathan Kline Institue, Orangeburg, NY; [2]Dept. of Rehabil. Med., Weill Med. Col. of Cornell Univ., NYC, NY

Abstract: A fundamental question in neuroscience is the significance of asymmetry, or cerebral lateralization, which allows functional specialization of bilateral brain regions. Lateralization is a multifaceted phenomenon occurring at both the structural and functional levels, and can be expressed as either a stable lifelong trait, or as a dynamic feature during behavior and development. Importantly, abnormal lateralization has been linked to neurological and psychiatric disorders. There is increasing evidence of cerebral lateralization in rodents related to synaptic plasticity and memory (olfaction: Cohen et al., 2015; audition: Marlin et al., 2015; hippocampus: Shipton et al., 2014). For example, in piriform cortex (PCX) of rats performing a two-alternative forced choice odor discrimination task, we demonstrated local field potential (LFP) beta frequency oscillations in the left PCX are more robust during initial learning than the right PCX. In contrast, the right PCX shows beta band enhancement at the late stages of learning while the left PCX changes return to baseline. The extent of this asymmetry in plasticity through the olfactory system, and its underlying mechanisms are unknown. Here using telemetry LFP recordings of rats performing odor discrimination task as before (Cohen et al., 2015), we demonstrate that the orbitofrontal cortex, a region receiving both direct and indirect input from the PCX, is also lateralized during the learning. However, as opposed to PCX where the left hemisphere changes most dramatically during early learning, trial-related beta oscillations in right OFC are most pronounced during initial and reversal learning, with only limited change in left OFC.We are currently screening PCX and OFC for lateralization in molecular markers related to plasticity to begin to understand the mechanisms of this widespread cortical asymmetry related to odor learning.

Disclosures: **Y. Cohen:** None. **D. Putrino:** None. **D.A. Wilson:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.10/N47

Topic: D.01. Chemical Senses

Support: NIDCD NRSA F31 1F31DC014615-01A1 (K.S.C.)

NSF Grant IOS-1121471 (D.W.W.)

Alzheimer's Association 14-305847 (D.W.W.)

Title: Investigating attentional modulation of odor coding in the olfactory tubercle

Authors: ***K. S. CARLSON**, E. S. DAUSTER, M. A. GADZIOLA, D. W. WESSON; Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Depending on the internal state of an individual, the same sensory stimulus may be perceived differently at different times. Attentional processes are especially well known to allow an individual to filter out irrelevant stimuli to focus on aspects of the environment that are relevant for survival. What are the underlying neural processes that contribute to this difference in perception? While this question has been investigated at the cellular level in sensory systems such as vision and audition, very few studies have explored attentional modulation of olfaction at this level. Human functional imaging research has, however, uncovered evidence that odor-directed attention modulates the representation of odors in the olfactory tubercle. While the rodent olfactory system lends itself well as an ideal model to explore the influences of attention at the cellular level, behavioral tasks involving manipulations of selective odor-directed attention in rodents are unavailable. To overcome this, we designed a novel two-alternative choice behavioral task that yields precise and systematic manipulations of odor-directed selective attention. Our results to date 1) show the development and implementation of this novel task in Long-Evans rats, 2) provide fundamental insights into the role of attention in modulating olfactory acuity, and 3) demonstrate the feasibility of utilizing this task, paired with extracellular recordings, to investigate the effects of attentional modulation on odor coding in the olfactory tubercle. Together, these results enhance our understanding of how attention shapes sensory processing and perception, which is impaired in a variety of neurological disorders, including Alzheimer's disease.

Disclosures: **K.S. Carlson:** None. **E.S. Dauster:** None. **M.A. Gadziola:** None. **D.W. Wesson:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.11/N48

Topic: D.01. Chemical Senses

Support: NIH Grant DC008855

NIH Grant DC004657

NIH Grant DC000566

Title: Role of basal forebrain cholinergic neurons in olfactory learning

Authors: *A. F. NUNEZ-PARRA, D. RESTREPO;
Cell and Developmental Biol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: The ability of the olfactory system to represent sensory cues is strongly influenced by neuromodulators released in response to a challenging and constantly changing external environment. Of particular interest is the neuromodulator acetylcholine (ACh), which has been linked in several brain regions with attention, cue detection and learning. The olfactory bulb and piriform cortex receive abundant cholinergic innervation from the basal forebrain and it has been suggested that in these regions ACh promotes contrast enhancement among similar odorants and network synchronization to generate a more efficient olfactory coding, ultimately affecting olfactory learning and memory. Substantial efforts have been made to study the cellular effects of ACh in the olfactory system, yet the time course of activation of basal forebrain neurons during active olfactory learning remains unknown. Here, we performed multielectrode recordings from the basal forebrain of awake freely moving animals exposed to the associative learning paradigm go-no go. This task studies learning of a water-deprived rodent to actively discriminate between two odorants (a rewarded and a non-rewarded odor). We found that more that 50% of the basal forebrain neurons activity diverges (either increase or decrease their firing rate) when the animal started the behavioral trial. Importantly, when the stimulus was presented, between 18 and 32% of the basal forebrain neurons exhibited a change in firing rate. During HIT trials the increase/decrease in activity was long lasting (4s), extending after the end of stimulus delivery (delivery time=2.5s). In contrast, when the animal rejected a trial correctly, the modulation in firing rate was transient and lasted only 1.5s. This condition was not maintained in a relatively easy task when the stimulus consisted of an odor vs. mineral oil or in a go-go task, where both stimuli are rewarded and no discrimination between stimuli is required. In addition, we used selective optogenetic stimulation to identify neurons that were directly or indirectly activated by light in the basal forebrain of animals expressing channelrhodopsin under the control of the choline acetyltransferase promoter, an enzyme that is exclusively expressed in cholinergic neurons. Our preliminary results show that these neurons are recruited at the

beginning of the trial and after the odors are presented. In summary, our data shows for the first time in awake freely moving animals that precise temporal cholinergic release from the basal forebrain plays an important role in information processing required for proper olfactory coding.

Disclosures: A.F. Nunez-Parra: None. **D. Restrepo:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.12/O1

Topic: D.01. Chemical Senses

Support: NIH/NIDCD R21 DC012014

NIH/NIDCD R01 DC010014

NIH/NIDCD R01 DC013243

NSF GRFP

Title: Odor-driven oscillations in human olfactory cortex

Authors: *H. JIANG[1], S. LEVINE[1], S. SCHUELE[1], J. ROSENOW[1], J. PARVIZI[2], J. TAO[3], J. GOTTFRIED[1];
[1]Neurol., Northwestern Univ., chicago, IL; [2]Stanford Univ., Palo Alto, CA; [3]Neurol., The Univ. of Chicago, Chicago, IL

Abstract: The mechanisms by which human olfactory cortex processes odor stimuli are poorly understood. Previous studies involving human intracranial EEG have shown that the amygdala produces event-related potentials in response to odorants, but the electrophysiological properties of important olfactory regions such as piriform cortex are not yet known. Five patients with depth electrode coverage in medial temporal lobe (including piriform cortex) performed an odor detection task while undergoing intracranial EEG monitoring. Each patient received between four and eight odors varying across dimensions of valence and edibility, along with odorless air, and on each trial reported whether or not an odor was detectable. In all five patients, results showed that piriform cortex exhibited significantly increased power in the theta-band frequency (4-7 Hz) after the onset of sniff during odor presentation relative to no odor presentation. We then applied a linear binary classifier to trial-wise piriform spectrograms, which reliably predicted the specific odor presented on each trial 55-75% of the time (chance = 50 %). Classification accuracy across odor pairs was also higher among piriform electrodes relative to other electrodes placed within cortical and subcortical regions, ranking within the top 1-10% of recorded electrodes. These results confirm involvement of posterior piriform cortex in early processing of odor identity. Additionally our finding of prominent theta-band odor-evoked

activity accords well with animal models implicating theta rhythms as a fundamental olfactory electrophysiological signature.

Disclosures: **H. Jiang:** None. **S. Levine:** None. **S. Schuele:** None. **J. Rosenow:** None. **J. Parvizi:** None. **J. Tao:** None. **J. Gottfried:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.13/O2

Topic: D.01. Chemical Senses

Support: HFSP CDA00029/2013-C

EU MARIE CURIE CIG DOPAPREDICT

Title: Odor source localization in a single sniff

Authors: ***S. HAESLER**, J. ESQUIVELZETA-RABELL, K. MUTLU, J. NOUTEL; Neuroelectronics Res. Flanders, Leuven, Belgium

Abstract: Navigation, finding food sources and avoiding danger critically depend on the identification and spatial localization of airborne chemicals in the environment. When monitoring the olfactory environment, rodents engage in active sampling behaviours, commonly also referred to as exploratory sniffing. Exploratory sniffing is characterized by stereotypical high frequency respirations, which are also reliably evoked by novel odorant stimuli. To resolve the mechanistic principles underlying exploratory sniffing and novelty processing, we have developed a behavioral paradigm in mice, which provides parametric measures of novelty perception. In our task, we present novel and familiar olfactory stimuli to head-restrained mice while measuring respiration. To enable highly reliable, repeated monitoring of respiration in the same animal over long time periods, we developed a novel method for non-contact respiration measurements using infrared thermography. Using video frame analysis, we extract respiration rates from the thermal data, collected at 60Hz. By simultaneously measuring intranasal pressure through implanted cannula, we validate our novel method and confirm faithful detection of inhalation and exhalation onsets. Moreover, we find that the common practice of cannula implantation, distorts odor flow and creates an asymmetric stimulus bias between the two nostrils. One key feature of our novel method is the ability to retrieve positional information about the location and movement of the two nostrils. When introducing novel odorants, we find a remarkable degree of nostril movements, even though mice are head-restrained. Using asymmetric stimulus delivery, we further find that mice direct their nostrils to the odor source within the first sniff after odor onset. This olfactomotor response is highly reliable, as mice orient towards the correct side in >80% of first novel odor presentations. Directional responses

habituate concurrently with subsequent odor presentation, similar to the habituation of the sniffing response. The spatial orienting response provides direct evidence mice can use intranasal stimulus gradients for source localization. In our ongoing work, we investigate the effect of unilateral naris occlusion on the spatial orienting response and explore which brain areas are critical for odour source localization.

Disclosures: **S. Haesler:** None. **J. Esquivelzeta-Rabell:** None. **K. Mutlu:** None. **J. Noutel:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.14/O3

Topic: D.01. Chemical Senses

Title: Experimental platform for dissecting olfactory system in *C. elegans*

Authors: ***K. ASHIDA**, H. SHIDARA, K. HOTTA, K. OKA;
Keio Univ., Yokohama, Japan

Abstract: The sensory neuron, AWC, responds to removal of odor stimulus in Caenorhabditis elegans. AIY interneurons, which receive inhibitory synaptic inputs from AWC, respond to the onset of odor stimulus. Although these neurons have been known to relate to olfactory learning and adaptation, the relation between AWC and AIY neurons on olfactory information processing and learning is poorly understood. To quantify this relation directly, timing and intensity of neuronal activity are crucial. Therefore, simultaneous Ca2+ imaging of AWC and AIY is a powerful tool. First, we prepared transgenic worms to dissect two neurons. Because Ca2+ responses of AIY interneurons to olfactory stimulus are detected only in neuritis close to axon of AWC, it is difficult to distinguish neural activities in neurites of AIY from that in axon of AWC. Therefore, we expressed the Ca2+ indicator with nuclear localization tag in AWC and the conventional indicator in AIY [Schrodel, et al., 2013], and checked Ca2+ responses to the odor, isoamyl alcohol, in these neurons. The Ca2+ in AWC increased with odor removal and decreased with odor application. On the other hand, AIY responded to odor application with Ca2+ increasing. These responses correspond to the result from the previous research [Chalasani, et al., 2007], and indicate that the simultaneous imaging system works correctly. Then, we identified the relation between Ca2+ responses from AWC and AIY using this system. AIY neurites responded before AWC soma response. In AWC, the onset of Ca2+ increase started axon first, and soma and dendrites follow it. Moreover, we found new characteristics of Ca2+ responses in AWC. AWC has been known to be stationary against odor stimuli [Kato, et al., 2014]. However, Ca2+ responses to periodic stimuli of odor were not stationary. There was a delay from stimulus timing to the outset of Ca2+ responses. The delay time at the onset of stimuli was shorter than

that after a few minutes from the onset of the stimulus. In conclusion, we succeeded to construct simultaneous imaging system of the sensory neuron and interneuron. This system clarified the timing of the Ca2+ response of AWC and AIY. The result showed that the system was useful for elucidating the olfactory information processing, especially adaptation and learning.

Disclosures: **K. Ashida:** None. **H. Shidara:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.15/O4

Topic: D.01. Chemical Senses

Support: Fondecyt 1141233 (Chile)

Title: Unraveling the dual role of the Dopaminergic system underlying locomotor behavior and the innate value of an aversive olfactory stimulus in *Drosophila*

Authors: N. FUENZALIDA-URIBE, *J. M. CAMPUSANO;
P. Univ. Catolica Chile, Santiago, Chile

Abstract: The Mushroom Body (MB) is a brain integration center key in processing several sensory stimuli and in defining locomotor behavior in insects. Dopamine (DA) systems innervate and modulate the activity of neurons thorughout the entire insect brain including the MB. It has been shown that through the regulation of specific neuronal populations in MB, this biogenic amine becomes relevant in the modulation of different innate behaviors in *Drosophila*. Here we have studied the role of two DArgic clusters (PAM and PPL1) which innervate different zones of MB, on the innate value observed in adult flies in presence of an aversive stimulus (Benzaldehyde, Bz) and the locomotor behavior associated. In order to do this, we manipulated the synaptic transmission of these DA neural clusters through the expression of Tetanus toxin (Tetx), Kir2.1 channel and TrpA1 channels. Our results show that PPL1 and PAM neurons differentially modulate the innate value of Bz in the adult fly. On other hand, blocking the neurotransmission of PAM neurons also decreased the locomotor behavior of flies exposed to Bz, an effect not observed when silencing PPL1. A chronic Nicotine treatment rescued the defect on motor output observed in PAM-silenced neurons while did not rescue the changes in the innate value to Bz. Our results show the differential contribution of specific DArgic pathways innervating MB in the modulation of locomotor behavior and the innate value to an odorant in *Drosophila*. Supported by Fondecyt 1141233. OBJ OBJ OBJ OBJ OBJ OBJ OBJ OBJ OBJ

Disclosures: **N. Fuenzalida-Uribe:** None. **J.M. Campusano:** None.

Poster

326. Olfaction: Behavior, Perception, and Neurophysiology

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 326.16/O5

Topic: D.01. Chemical Senses

Support: NIH R01 DC013071

Title: Alterations in brain-derived leptin-homolog lead to obesity phenotypes in *Drosophila* through regulation of food odor value signaling

Authors: *J. BESHEL, Y. ZHONG;
Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Motivated feeding results from the interplay of homeostatic and hedonic drives and dysregulation of either may contribute adversely to conditions of overweight and obesity. Recent work demonstrates domeless receptors in *Drosophila* can be activated by human Leptin, a typically adipose-derived “satiety hormone” with a long-established role in weight regulation. Interestingly, knockdown of endogenous domeless-ligand upd2 in the fat body of flies leads to smaller body size and has no effect on feeding, the opposite behavior of leptin-deficient mammals. While we replicate the lower weight and unchanged food intake in upd2-manipulated flies, we further show manipulations to another endogenous ligand for the domeless receptor, brain-based unpaired 1 (upd1), recapitulate mammalian obesity phenotypes in flies. Flies with reductions in upd1 restricted to neural tissue show increased weights, increased food intake, and increased attraction to food odors. We additionally report domeless receptors likely mediate observed phenotypes. We show behavior-relevant domeless receptors are located on neurons expressing *Drosophila* Neuropeptide F (dNPF), the Neuropeptide Y (NPY) homolog, in the central brain with targeted receptor knockdown specifically to these cells replicating increased weight, attraction and intake phenotypes. We speculate upd1 acts as the homeostatic regulator of our previously reported dNPF food odor value signal, up- or down-regulating this hedonic signal as a function of satiety state. Our findings suggest Leptin-NPY and upd1-dNPF represent functionally homologous circuits across diverse species and imply in mammals adipose- and less understood brain-derived Leptin may play different roles in feeding and weight regulation.

Disclosures: J. Beshel: None. **Y. Zhong:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.01/O6

Topic: D.01. Chemical Senses

Support: NIDCD Grant RO1DC013904

Title: Changes in taste and odor preference following bariatric surgery in humans

Authors: H. KITTRELL[1], W. GRABER[2], *J. D. SAMMONS[1], K. CZAJA[3], A. HAJNAL[4], P. M. DI LORENZO[5];
[1]Psychology, Binghamton Univ., Binghamton, NY; [2]St. Joseph's Hosp. Hlth. Ctr., Syracuse, NY; [3]VB and DI, Univ. of Georgia, Athens, Athens, GA; [4]Neural and Behavioral Sci., Penn State Univ., Hershey, PA; [5]Psychology, BInghamton Univ., Binghamton, NY

Abstract: To achieve long-term weight loss in obese subjects, reverses enhanced preference and intake of sweet/fatty foods. Although taste preference changes following bariatric surgery have been previously described, potential concomitant odor preference changes remain unexplored. Therefore, the aim of this study was to determine the relationship between taste and odor preference changes and successful weight loss following bariatric surgery in a cohort of patients by means of a questionnaire. The study was performed on 57 human subjects with body mass index (BMI) above 30 (at least class I obesity), who were scheduled to receive or had previously received Roux-en-Y gastric bypass (RYGB) surgery or the gastric sleeve procedure. A Self-Assessment Manikin (SAM) test was used to measure the participant's affective reaction (ranging from pleasure to displeasure) to a variety of food-related and odor-related pictures. An unpaired t test was performed to determine the difference between SAM ratings pre- and post-surgery; Spearman rho was used to describe the relationship between amount of change in BMI since surgery and SAM ratings. Results confirmed earlier reports about changes in sweet/fatty foods preference after surgery and revealed a shift in preference toward less calorie-dense foods. Patients experiencing calorie-dense foods aversion revealed more postoperative weight loss and reduction in BMI compared to their counterparts without these features. Our results also revealed that changes in olfactory preferences that occur following bariatric surgery do have a significant correlation with successful post-surgical change in BMI. Importantly, data showed a clear and consistent association of coffee and banana odor ratings and the change in BMI following surgery. In particular, patients rating a coffee and/or banana odor as more pleasing after surgery had a lower post-surgical BMI. Coffee odor was also shown to become more pleasing as time since surgery increased. In conclusion, results showed that following bariatric surgery both taste and odor preferences are significantly altered and that these changes correlate with more successful changes in BMI. These results may suggest diagnostic criteria to identify people at risk for less than optimal changes in BMI following bariatric surgery.

Disclosures: H. Kittrell: None. **W. Graber:** None. **J.D. Sammons:** None. **K. Czaja:** None. **A. Hajnal:** None. **P.M. Di Lorenzo:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.02/O7

Topic: D.01. Chemical Senses

Support: NIH Grant R01DC00882

NIH Grant P30DC011735

NIDCD Grant T32DC00014

Title: Mice selected for high and low saccharin intake differ in consumption of appetitive taste solutions regardless of their taste quality

Authors: *R. POOLE[1], M. INOUE[2], N. BOSAK[1], A. BACHMANOV[1];
[1]Monell Chem. Senses Ctr., Philadelphia, PA; [2]Tokyo Univ. of Pharm. and Life Sci., Hachioji, Tokyo, Japan

Abstract: In mice, variation in sweet taste is influenced by polymorphisms of the Tas1r3 gene. However, there are Tas1r3-independent genetic factors that influence variation in sweet taste responses as well. To examine Tas1r3-independent sweet taste mechanisms, we selectively bred two strains of mice that share a common Tas1r3 allele, yet differ in saccharin intake: the Sac-H and Sac-L strains, with high and low saccharin intakes, respectively. Electrophysiological recordings of taste-evoked responses in the chorda tympani nerve showed that these strains had similar peripheral taste responsiveness. These results suggest that strain differences in saccharin intake may be caused by strain differences in central taste processing. To distinguish whether these central mechanisms are specific to sweet taste or are more general and apply to other appetitive taste stimuli, we examined preferences for sweet (saccharin, sucralose, and sucrose) and non-sweet (oil, maltodextrin, MSG, and IMP) palatable solutions using two-bottle choice tests. We found that regardless of taste quality, when preference scores exceeded 80% in both strains, Sac-H mice consumed significantly more taste solution than Sac-L mice. Interestingly, although mice preferred MSG, preference scores did not exceed 80% at any concentration tested, and MSG intakes did not differ between strains either. Given that MSG and IMP have the same taste quality (i.e., umami), these results support the conclusion that differential consumption of taste solutions by Sac-H and Sac-L mice is driven by the hedonic value of a taste solution, not taste quality. Our data suggest that mice selected for saccharin intake are a valuable model for analyzing mechanisms of reward because taste stimuli must evoke a strong appetitive response to trigger differential intake.

Disclosures: R. Poole: None. **M. Inoue:** None. **N. Bosak:** None. **A. Bachmanov:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.03/O8

Topic: D.01. Chemical Senses

Support: Ajinomoto Co., Inc.

Title: Generalization of conditioned taste aversion of dashi to salts is modified by lactic acid in mice

Authors: *E. R. DELAY[1], B. M. WEAVER[2], D. R. LANE[2], T. KONDOH[3];
[1]Dept. of Biol., [2]Neurosci. Undergraduate Program, Univ. of Vermont, Burlington, VT; [3]Inst. for Innovation, Ajinomoto Co., Inc., Kawasaki 210-8681, Japan

Abstract: Dried bonito *dashi*, a Japanese fish stock, is an important component of Japanese cuisine and is a preferred flavor of humans and rodents. It is made of a complex mixture of amino acid, proteins, organic acids and minerals. Previous research (Delay & Kondoh, 2015) reported that in mice *dashi* elicits all 5 of the basic tastes. The purpose of this study was to use conditioned taste aversion (CTA) methods to determine if an aversion to *dashi* generalizes to one or more of 4 salts (NaCl, KCl, CaCl2, MgCl2), and if lactic acid (a large component of *dashi*) to these salts alters the taste and resulting generalized aversion in C57BL/6J mice with compromised olfactory systems. Conditioning and generalization testing were done with 25% solution of *dashi* (conditioned stimulus) presented in a Davis Rig (MS160). Stimulus generalization was measured by counting licks when mice were presented with NaCl (100 and 300 mM), KCl (100 and 300 mM), CaCl2 (15 and 30 mM), and MgCl2 (20 and 40 mM), with or without 0.9% lactic acid added. We found that all 4 salts showed mild naturally aversive qualities at their highest concentration. Additionally, *dashi* CTA generalized more to the divalent salts than the monovalent salts. Interestingly, lactic acid had little effect on CTA generalization to the monovalent salts whereas it decreased generalization to divalent salts. The CTA of *dashi* did not generalize to lactic acid alone. These results indicate that all 4 salts may contribute to the taste of *dashi* and that interactions between lactic acid and divalent salts alter the tastes elicited by these salts. These findings suggest lactic acid may play a complex role on the perception of foods. Further studies will examine whether citric acid, another organic acid, can also modulate CTA generalization in a manner similar to lactic acid.

Disclosures: E.R. Delay: None. **B.M. Weaver:** None. **D.R. Lane:** None. **T. Kondoh:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.04/O9

Topic: D.01. Chemical Senses

Support: NSF IOS-0951016

Title: Multiple glutamate receptors detect L-amino acid taste in mice

Authors: *S. PAL CHOUDHURI, R. J. DELAY, E. R. DELAY;
Biol., The Univ. of Vermont, Burlington, VT

Abstract: G-protein coupled receptors (GPCRs) are thought to be involved in the detection of umami and possibly L-amino acid taste. These include the heterodimer T1R1/T1R3, mGluR4, and mGluR1. While several studies suggest T1R1/T1R3 is a broadly tuned L-amino acid receptor, little is known about the function of mGluRs in L-amino acid taste transduction. Our previous data indicate that other taste receptors may also contribute to the taste of L-amino acids and 5' nucleotides such as inosine 5' monophosphate (IMP). Here we performed calcium imaging of isolated taste sensory cells (TSCs) using the ratiometric dye Fura 2 AM to investigate the role of different mGluRs in detecting various L-amino acids. We harvested TSCs from wild type (C57BL/CJ) and T1R3 knock-out (T1R3 KO) mice. Using selective agonist for various mGluRs such as (RS)-3, 5-dihydroxyphenylglycine (DHPG) (an mGluR1 agonist) and L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) (an mGluR4 agonist), we evaluated the responses of TSCs to determine if each TSCs might respond to IMP and to the L-amino acids, monopotassium L-glutamate, L-serine and L-arginine. Additionally we used selective antagonists against different mGluRs such as (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA) (an mGluR1 antagonist), (RS)-α-methylserine-O-phosphate (MSOP) and (RS)-α-cyclopropyl-4-phosphonophenylglycine (CPPG) (both mGluR4 antagonists) to determine if the antagonists can block the responses elicited by these L-amino acids and IMP. We found that antagonist for mGluR4 and mGluR1 significantly blocked the responses elicited by IMP and each of these L-amino acids. Additionally the antagonists were able to block L-amino acid elicited responses in TSCs of T1R3 KO mice. Collectively, this study provides evidence for the involvement of several mGluRs in L-amino acid taste responses in mice and supports the hypothesis that multiple receptors contribute to IMP and L-amino acid taste.

Disclosures: S. Pal Choudhuri: None. **R.J. Delay:** None. **E.R. Delay:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.05/O10

Topic: D.01. Chemical Senses

Support: NIH NIDCD Grant DC014428

Title: Receptive field size, chemical and thermal responses and fiber conduction velocity of rat chorda tympani geniculate ganglion neurons

Authors: Y. YOKOTA, *R. M. BRADLEY;
Univ. Michigan Sch. Dent., Ann Arbor, MI

Abstract: Afferent chorda tympani (CT) fibers innervating anterior tongue taste receptors in fungiform papillae have neuron cell bodies in the geniculate ganglion, that differ in soma size and axon diameter. This structural diversity could relate to functional differences. To investigate these functional differences we recorded extracellular responses from single geniculate ganglion neurons to lingual application of chemical and thermal stimuli and determined receptive field properties. Receptive field size was mapped by electrical stimulation of individual fungiform papillae. Response latency to electrical stimulation was used to determine fiber conduction velocity. To date we have analyzed the responses of 30 neurons to room temperature chemical stimuli (0.5M NaCl, 0.5M NH4Cl, 0.01N HCl, 0.03M citric acid, 0.02M quinine HCl and 1.0M sucrose), and distilled water at 4°C. Based on response to chemical and thermal stimuli, neurons were divided into five categories. Three neurons were classified as SALT, responding only to NaCl and NH4Cl. Average receptive field size of these neurons was 6.3 ± 1.3 papillae. Seven neurons, classified as OTHER, responded to salts and other chemical stimuli (acids, bitter and sweet) and had smaller receptive fields (4.5 ± 1.3 papillae). Six neurons responded to salts and cold, classified as SALT/THERMAL, and 7 neurons responded to salts, other chemical stimuli and cold, classified as OTHER/THERMAL. Average receptive field size of SALT/THERMAL was 6.4 ± 1.7 papillae, and for OTHER/THERMAL was 5.1 ± 1.9 papillae. Seven neurons categorized as THERMAL, responded only to cold. Receptive field size of these neurons was 1.6 ± 0.5 papillae, significantly smaller than average field size of SALT and OTHER, and located at the tongue tip. These data show that neuron response categories have different receptive field sizes. For all 30 neurons, receptive field size ranged from 1 to 8 papillae. Conduction velocity was determined using latency to electrical stimulation (46.8 ± 13.1 msec) and conduction distance (58 ± 3 mm). The conduction velocity of 29 neurons was 0.4-2.0 m/s, classified as C fibers, and one neuron was classified as an Aδ fiber (2.5 m/s). Neurons with large receptive fields had higher conduction velocities than neurons with small receptive fields. We conclude that geniculate ganglion neurons can be distinguished by receptive field size and response properties. Because several papillae and taste buds are connected to a single ganglion neuron, responses from the periphery reflect broad taste responsiveness from multiple taste organs.

Disclosures: Y. Yokota: None. **R.M. Bradley:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.06/O11

Topic: D.01. Chemical Senses

Support: Graduate Research and Creative Activity Award, University of Nebraska at Omaha

Title: Sex differences in rat glossopharyngeal nerve taste responses

Authors: L. J. MARTIN[1], *S. I. SOLLARS[2];
[1]Psychology, [2]Univ. of Nebraska at Omaha, Omaha, NE

Abstract: Sex differences in taste-guided behavior have been well established in several species, but the gustatory processes underlying these behaviors have not been well characterized. While male and female rats display differences in NaCl preference and discrimination, there is contradictory evidence whether the chorda tympani nerve (CT) responds differently to NaCl based on sex. Additionally, it is unclear whether sex differences exist in peripheral processing of sweet and bitter stimuli, though electrophysiological recordings from the parabrachial nucleus of the pons indicate differential activity toward these two stimuli in male versus female rats. To further characterize the role of sex differences in taste processing, whole-nerve electrophysiology was performed on the glossopharyngeal nerve (GL) of adult male and female Sprague-Dawley rats in response to taste stimulation of the posterior tongue. Stimuli included: 0.5 M sucrose, 0.1 M citric acid, 0.01 M quinine, 0.05-1.0 M NaCl, and 0.05-1.0 M sodium acetate (NaAc). Responses to all stimuli were compared to a 0.5M NH_4Cl reference standard, with 15 sec of steady state responses starting 10 seconds after stimulus onset used as the data. Water rinses occurred before and after each stimulus. After these stimuli were administered, a 100 μM solution of the epithelial sodium channel blocker amiloride was applied to the posterior tongue and the NaCl and NaAc series were completed again using the amiloride solution as the rinse. Surprisingly, male but not female responses to NaCl and NaAc were significantly suppressed by amiloride. Results also indicate that GL responses to sucrose were higher in males than in females. This is consistent with previous research indicating that males have a lower discrimination threshold for sucrose. Previous work indicates that glossopharyngeal nerve responses are not sensitive to amiloride, but our results suggest that salt transduction on the posterior tongue may be different between males and females. This result could explain the sex-related differences in behavioral responses to NaCl, as amiloride-sensitive and amiloride-insensitive salt pathways have been shown to have different roles in regulating behavior. There were no significant differences in GL responses for the other solutions tested. Overall, our results suggest that taste input from the posterior tongue is processed differently between male and female rats, and these differences may contribute to taste-guided behavior.

Disclosures: L.J. Martin: None. **S.I. Sollars:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.07/O12

Topic: D.01. Chemical Senses

Support: DGAPA/UNAM IN200413

Title: Perinatal undernutrition alters feeding behavior and fat c-Fos expression in the solitary tract neurons of adult male Wistar rats

Authors: L. RUBIO NAVARRO[1], *M. A. SALAS[3], M. REGALADO[2], C. TORRERO[2]; [1]Developmental Neurobio. and Neurophysiol., [2]Natl. Univ. of México, Queretaro, Qro, Mexico; [3]UNAM, Queretaro, Mexico

Abstract: Malnutrition early in life has long lasting effects on brain areas related with food intake. Food selection can be affected by undernutrition, and with self-selection method we can evaluate qualitative changes in nutritional balance. The present study was design to evaluate the alteration on feeding preferences patterns using protein, carbohydrate and fat in adult rats that were exposed to perinatal caloric restriction. Additionally, the estimation of the neuron activated by fat stimuli in the nucleus of the solitary tract (NST) in adult male undernourished rats at present is unknown. We used male control (CG) and undernourished groups (UG) of 9 week old. In the undernourished group (UG) pregnant dams received different percentage of a balance diet during gestation. After birth pups continue the undernutrition by remaining 12h with a foster dam, and 12h with a nipple-ligated mother. At weaning, five pups were house in group until self-selection paradigm. Three days before testing were house individually and body weight and food consumption were evaluated. After that, during three days we recorded the protein, carbohydrate and fat intake and body weight of rats. After the test the male rat were food and water restricted during 12h, thereafter stimulated with fat and sacrificed 90 min post stimulation, controls were isolated until sacrifice and brainstem tissue was processed by c-Fos immunoreactivity and different areas of the NST were considered. We observed that undernourished rats consumed the same amount of carbohydrates than control rats, protein and fat intake tended to increase during the three days of testing compared with control rats. Control group showed little c-Fos like immunoreactivity (FLI) by contrast, fat food induce clear and consistent more FLI in medial and caudal areas of the NTS in undernourished rats compared with control subjects. The present data contribute to define basic neuronal network activated by gustatory cues affected by perinatal restriction that indicate the alteration in associative learning capacities and homeostatic adaptations to the environment, that make prone adult males to the development of abnormal feeding behavior.

Disclosures: L. Rubio navarro: None. **M.A. Salas:** None. **M. Regalado:** None. **C. Torrero:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.08/O13

Topic: D.01. Chemical Senses

Support: NIH Grant DC009982

NIH Grant DC014306

NIH Grant DC000011

Title: Organization inhibitory circuitry connected to projection neuron populations in the mouse rostral nucleus of the solitary tract

Authors: *J. A. CORSON, R. M. BRADLEY;
Univ. of Michigan, Ann Arbor, MI

Abstract: The rostral nucleus of the solitary tract (rNST) is the target of gustatory primary afferents from the oral cavity. Thus, the rNST is in a prime position to control all aspects of gustatory-related behavior, from perception (parabrachial nucleus [PBN] projections) to reflexes (reticular formation [RF] projections). However, the intrinsic circuitry that transforms incoming sensory information into output action potential trains remains relatively unstudied. Using mice expressing channelrhodopsin under the control of the vesicular GABA transporter promoter (VGAT-ChR2), we investigated the organization of local inhibitory connectivity. Fluorescent microbeads were injected into either the PBN or RF to retrogradely label neurons projecting to either of these second-order targets. Local GABAergic circuits were activated with a custom-made laser scanning photostimulation system while recording the resulting inhibitory synaptic currents (IPSCs) in an *in vitro* slice preparation. The laser spot was scanned over a grid (50 μm spacing, encompassing all rNST subdivisions) in a random order to systematically activate spatially discrete populations of inhibitory interneurons. IPSCs were automatically detected using a time-based deconvolution algorithm and the kinetics (amplitude, charge, rising slope, and decay constants) computed. Cells were filled with biocytin during recording and the cell morphology reconstructed following peroxidase visualization. The spatial distributions of inhibitory connectivity were compared using a magnitude-normalized linear distance algorithm. rNST-RF projection neurons received inhibitory connections from rNST regions further from the cell soma than rNST-PBN projection neurons. The total inhibitory charge from all stimulation sites was also greater for rNST-RF projection neurons. This suggests that neurons governing oromotor reflexes receive stronger local inhibitory modulation of firing patterns than those governing hedonics and perception. The dendritic morphology of rNST projection neurons as well as the optically-evoked synaptic amplitude and rising slope were only moderately correlated to the spatial distribution of inhibitory connectivity. Further, the strength (i.e. amplitude and charge) of optically-evoked IPSCs was larger than spontaneous IPSCs. This indicates a specificity of local inhibitory interneuron axon targeting rather than a stochastic connectivity and

that this local inhibition is stronger than extrinsic inhibition. Together, these results suggest the presence of distinct local circuits capable of modulating the diverse rNST output pathways.

Disclosures: J.A. Corson: None. **R.M. Bradley:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.09/O14

Topic: D.01. Chemical Senses

Support: NIDCD Grant DC006914

Title: Optogenetic manipulation of lateral hypothalamic input to the nucleus of the solitary tract modulates licking behavior in the awake rat

Authors: J. D. SAMMONS[1], C. E. BASS[2], J. D. VICTOR[3], *P. M. DI LORENZO[1]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]Dept. of Pharmacol. and Toxicology, Univ. of Buffalo SUNY Sch. of Med. and Biomed. Sci., Buffalo, NY; [3]Brain and Mind Inst., Weill Cornell Med. Sch., New York, NY

Abstract: The lateral hypothalamus (LH) sends direct synaptic connections to the nucleus of the solitary tract (NTS, the first synaptic relay in the central gustatory pathway). Through electrical stimulation, the LH has been shown to modulate gustatory neurons within the NTS (Matsuo et al., J. Neurosci. 4:1201-1207, 1984). However, the functional significance of direct synaptic connections from the LH to the NTS remains inconclusive. Here, we used optogenetic tools to selectively manipulate LH-NTS input while the rats were preforming a Go-no-Go (GnG) task to assess the role of the LH in modulating the lick cycle. Initially, we infused adeno-associated viral constructs encoding either Channelrhodopsin-2 (ChR2) or halorhodopsin (eNphR3.0) bilaterally into the LH of male rats. Following 2-4 wks, we implanted an optrode consisting of a fiber optic cable attached to a bundle of 8 tungsten microwires into the taste-responsive portion of the NTS and allowed the rats to recover. Rats were then water deprived and placed in an experimental chamber where they were allowed to freely lick in a GnG paradigm. A single cue stimulus lick (always 0.1 M NaCl) was presented followed by 5 dry licks and then 3 licks of a test stimulus (0.1 M NaCl, 0.1 M MSG/IMP or 0.1 M KCl). After a 1 s timeout, the task was to continue licking for a 3-lick (12µL/lick) 0.5 M sucrose reward if the test and cue stimuli were the same and to withhold licking if they were different. Continued licking when test and cue were different was punished by 3 licks of 1 mM quinine and a 5 s timeout. In a random half of each trial type, LH fibers in the NTS were optically stimulated (25 Hz of 473 nm or 532 nm laser light at 8-10 mW) for a maximum 1 s per test lick. Preliminary results show that optogenetic stimulation of LH-NTS input extended the inter-lick bout duration following a correct rejection. Additionally,

in some cases, the lick frequency was increased after incorrectly accepting the punishment stimulus. No significant differences were observed in reward presentation trials. These data suggest that LH input to the NTS may modulate the initiation and termination of lick bouts through its direct influence on NTS output.

Disclosures: J.D. Sammons: None. **C.E. Bass:** None. **J.D. Victor:** None. **P.M. Di Lorenzo:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.10/O15

Topic: D.01. Chemical Senses

Support: NIDCD grant RO1DC00691

Title: Transfer of chemosensory information between the NTS and PbN in the awake-behaving rats

Authors: *O. D. ESCANILLA, P. M. DI LORENZO;
Binghamton Univ., Binghamton, NY

Abstract: The nucleus of the solitary tract (NTS) is the first central nuclei relay in the gustatory pathway and has major projections to the parabrachial nucleus of the pons (PbN; the second synapse in the central gustatory pathway). Previous studies in anesthetized rats have shown that PbN cells initially follow the temporal pattern of NTS cells, but changes in the later portion of the response (Di Lorenzo and Monroe, Brain Res, 763 (2) 167 - 181, 1997). Here, we simultaneously recorded neurons from the NTS and the PbN to examine how the representation of chemosensory information is transferred between these two structures in awake, behaving rats. To do this, rats were implanted with an 8-tungsten wire electrode in both the NTS (AP: -15.3, ML: 1.8, with a 4 mm head tilt) and the PbN (AP: -12.5, ML: 1.6, with a 4 mm head tilt) and allowed to recover. Rats were then mildly water deprived and placed in the experimental chamber containing a lick spout for fluid delivery. Gustatory stimuli were 0.1M Sucrose, 0.1M NaCl, 0.01M Citric Acid, 0.0001M Quinine, and artificial saliva (AS). Odor and paired odor-taste stimuli were formulated by diluting each odorant, (0.01%) amyl acetate or (0.01%) acetic acid, in either AS (for retronasal odor only presentations) or different tastant solutions. Odor concentrations were below the detectable gustatory or somatosensory range in rats. Each odor, taste or taste-odor stimulus was presented for 5 consecutive licks separated by AS rinses that were on a variable ratio 5 schedule. Analyses of coherence and cross correlation functions of joint firing patterns revealed properties of lick information that could underlie the functional connectivity between the two structures. More specifically, preliminary results show that some

lick coherent cells in the NTS unidirectionally inhibit anti-lick cells in the PbN while others have reciprocal inhibition with PbN cells that were not lick coherent. These findings will help us elucidate the neural circuitry between these two brainstem structures and how they are correlated to ingestive behavior. Supported by NIDCD grant RO1DC006914 to PMD

Disclosures: O.D. Escanilla: None. **P.M. Di Lorenzo:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.11/O16

Topic: D.01. Chemical Senses

Support: NIDCD Grant RO1-DC006914 to PMD

Title: A lick-related circuit in the parabrachial nucleus of the pons in the awake, freely licking rat

Authors: *M. S. WEISS[1], P. M. DI LORENZO[1], J. D. SAMMONS[1], J. D. VICTOR[2]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]Neurol. and Neurosci., Weill Cornell Med. Col., New York, NY

Abstract: In the rodent central pathway for gustation, taste quality information from the periphery first synapses in the nucleus of the solitary tract (NTS), which in turn sends the bulk of its projections to the parabrachial nucleus of the pons (PbN). Electrophysiological recordings from both the NTS and PbN have revealed a proportion of cells, recorded alongside taste-responsive neurons, that do not respond to taste stimuli, leaving in question the functional role of these neurons. Many of these non-taste-responsive cells show activity that is strongly lick-related. Here, we examined the activity of small ensembles of neurons that show patterns of activity relating to an animal's licking behavior to reveal the functional microcircuitry that supports these various cell types within the taste-responsive portion of the PbN. In addition to lick-related cells, we also studied taste-responsive neurons, as they often show strong coherence with licking (Weiss et al., J Neurophysiol 111: 1655-1670, 2014). Initially, an 8-channel electrode bundle was surgically implanted into the PbN of male Sprague-Dawley rats. Following recovery and 22hrs of water-deprivation, rats were placed in an operant chamber with free access to a lick spout. Each lick produced 12µl of a taste stimulus for five consecutive licks and artificial saliva (as a rinse) on a variable-ratio 5 schedule in between stimulus presentations. Results from 18 rats identified 2 groups of neurons that fired in phase with licks: a) "Lick+" neurons showed peak firing rates at the lick (n = 99), and b) "Lick-" neurons showed peak firing rates during the inter-lick interval (n = 25). Additionally, some cells were anti-lick cells (n = 24) that fired vigorously during the interbout interval but became quiescent during the lick bout.

Analyses of cross-correlation functions (CCFs) produced evidence of a reciprocal inhibitory circuit between Lick+ and Lick- cells. Analyses of simultaneously recorded taste-responsive cells and Lick+ cells suggested a common input to both cells with additional unidirectional excitation of the lick cell by the taste cell. CCFs of simultaneous recordings of multiple anti-lick cells suggested that they were influenced by a common input. We show evidence of a circuit where anti-lick cells inhibit lick and taste-responsive cells. When a lick bout begins the anti-lick cells release lick and taste-responsive cells from inhibition leading to a lick-phase locked circuit. The most critical remaining question is what neural elements control the activity of the anti-lick cells to initiate the lick cycle.

Disclosures: M.S. Weiss: None. **P.M. Di Lorenzo:** None. **J.D. Sammons:** None. **J.D. Victor:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.12/O17

Topic: D.01. Chemical Senses

Title: CCK and calbindin in the primate parabrachial nucleus

Authors: *B. GEHRING[1], S. DE LACALLE[2];
[2]Biomed. Sci., [1]Ohio Univ., Athens, OH

Abstract: As a peptide hormone, cholecystokinin (CCK) mediates satiety by acting on the CCK receptors distributed widely throughout the central nervous system, including the brainstem. Among other actions, CCK's stimulatory effects on the vagus nerve oppose those of ghrelin. Calbindin-D28k, which was also first shown to be present in the intestine, is expressed in a number of neuronal and endocrine cells. For example, CCK/calbindin-containing GABAergic neurons have been identified in the hippocampal formation. Anatomical interactions between calbindin-containing neurons and CCK fibers have been demonstrated in the rat thalamus (Battaglia, 1992). Following earlier work using CGRP as a marker for ascending visceral pathways in the human brain (de Lacalle & Saper, 2000) here we describe the distribution of calbindin- and CCK-immunoreactive elements in the primate parabrachial nucleus (PB), a pontine structure with a crucial role in autonomic control. Our observations were made on horizontal sections through the brainstem from two neurologically normal human individuals and two male Cebus monkeys. Tissue was processed for immunocytochemistry using commercially available antibodies. We found several areas of dense peptide immunoreactivity in fibers, as well as scattered stained cell bodies. The distribution of peptide-stained fibers was strikingly conserved compared with that described in the rat. The Cebus monkey's PB showed calbindin-positive neurons in the rostral sections of both the MPB and LPB, disappearing in the caudal

sections, which contained mostly fibers in the MPB. The human sections showed an abundance of calbindin-positive neurons in the rostral MPB and dense groupings of stained cell bodies in the MPB and LPB at caudal levels. By contrast, CCK-immunoreactive cell bodies were found at all levels throughout the human MPB and LPB but only in the most rostral sections of the Cebus LPB. In the Cebus, the MPB and LPB at the rostral levels contained also a dense network of traversing CCK-immunoreactive fibers. Compared with the literature on the functional anatomy of the PB and its afferent and efferent projections, our results contribute to define the chemical identity of these neuronal groups in the primate brain, thus providing support to the delineation of physiological roles for the distinct subnuclei of this crucial visceral regulatory region.

Disclosures: B. Gehring: None. **S. de Lacalle:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.13/O18

Topic: D.01. Chemical Senses

Support: SNSF Fellowship: P2GEP3_151816

NIDCD Grant:R01-DC010389

Title: Representation of multisensory signals in the gustatory cortex of rats before and after associative learning

Authors: *R. VINCIS, A. FONTANINI;
Dept. of Neurobio. and Behavior, SUNY at Stony Brook Univ., Stony Brook, NY

Abstract: Taste experience is inherently multimodal and strongly modulated by expectations. Most research on the effects of expectation on gustatory cortical (GC) activity has relied on using auditory cues. While auditory stimuli prove to be effective for establishing cue-responsiveness in GC, it is likely that in natural settings cues from other sensory modalities would be more ecologically relevant to taste. Indeed, in the context of foraging and eating images, odors, and tactile stimuli are more inherently associated with taste than sounds are. Therefore, GC neural activity may also represent anticipatory cues from these sensory modalities. In order to investigate this hypothesis, we recorded the activity of GC neurons in response to olfactory (O), visual (V), auditory (A), somatosensory (S), and gustatory stimuli before and after cue-taste association in alert rats. Adult rats (n=10) were chronically and bilaterally implanted with movable bundles of electrodes in GC. In a first group of naive subjects (untrained; n=5), orofacial movements and single unit activity (n=135) were recorded while sensory stimuli were randomly delivered. A second group (trained; n=5) was classically conditioned with four sensory stimuli (O, V, A and S) predicting the availability of sucrose.

During the entire training period (14 days) orofacial movements were recorded in order to ascertain the time-course of conditioning. At the end of the training, single unit responses to the conditioned cues were recorded from GC (n=118). Our results show robust multimodality of GC neurons in untrained rats (% neurons responding to O, S, V, and A, respectively: 14, 13, 5, 5). The majority of neurons were responsive to 1-stimulus modalities, with odor and somatosensory stimuli being the most effective. In the conditioning group, rats learned all the cue-taste pairs. Analysis of orofacial movements revealed that rats learned the cue-taste association faster with odor and somatosensory cues compared to visual and auditory ones. Learning led to a 2-fold increase in the proportion of responsive neurons for each cues and to an increase in the percentage of neurons responding to multiple cues. Further analyses showed that learning increase the similarity between responses to olfactory and somatosensory cues relative to visual and auditory ones. Altogether, our results show that GC neurons respond to stimuli from multiple sensory modalities both naively and when they are taste-predictive cues, that the neural representations' strength and similarity change with learning, and sensory modalities more ecologically related to gustation are associated with a taste faster and are similarly represented in GC.

Disclosures: **R. Vincis:** None. **A. Fontanini:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.14/O19

Topic: D.01. Chemical Senses

Support: NIDCD R01-DC012543

Title: Dynamic modulation of gustatory thalamocortical synaptic inputs by basolateral amygdala

Authors: ***M. E. STONE**, A. MAFFEI, A. FONTANINI;
Neurobio. and Behavior, SUNY Stony Brook, Stony Brook, NY

Abstract: Gustatory cortex (GC) neurons process multiple aspects of a tasting experience, encoding not only the physiochemical identity of tastes but also their anticipation and hedonic value. Information pertaining to these stimulus features reaches GC via the gustatory thalamus (VPMpc) and basolateral amygdala (BLA). However, it is not known if these inputs converge onto the same neurons or if limbic activity can affect evoked thalamocortical responses in GC. We have shown previously using intracellular recordings in GC that BLA stimulation evokes a monosynaptic EPSP followed by longer lasting inhibition. Train stimuli can maximally evoke excitation or inhibition depending on frequency and background state of the cell. The time-varying nature of the response suggests BLA may gate other GC inputs by altering the

excitatory/inhibitory balance. Here, we use *in vivo* intracellular recordings in anesthetized rats to describe the thalamocortical synapse and test our hypothesis that BLA can modulate cortical responses to VPMpc. Electrical stimulation of VPMpc evokes a monosynaptic, glutamatergic EPSP in all GC cells, followed 50/50 by either a multisynaptic, GABAergic inhibition or by a sustained depolarization. This response heterogeneity is consistent with the reported heterogeneity in GC responses to tastes and is enhanced when trains of VPMpc stimuli are delivered. Synaptic inputs from both VPMpc and BLA converge onto a large subpopulation of GC neurons. Comparing the dorsoventral profile of recording electrode sites, VPMpc-responsive cells are more dorsal while BLA-responsive cells are more ventral, with an area of significant overlap across GC. We confirm the presence of this convergence of inputs using anterograde tracing methods. GC cells receiving both monosynaptic inputs were used to examine the influence of BLA on VPMpc responses. Single shocks or 20 Hz BLA bursts preceded single VPMpc stimuli at varying latencies. Both BLA stimulation protocols can modulate the amplitude of the evoked VPMpc PSP at short latencies. The effect is mostly to suppress and less frequently to enhance VPMpc responses, a result consistent with the presence of mixed reversal potentials early in the evoked BLA PSP and also with the heterogeneity of VPMpc responses. The inhibitory tail of the BLA burst significantly decreases the variability of the GC neuron's membrane potential for up to 250 ms, a possible mechanism for BLA to influence GC taste response variability. In summary, our results show that thalamic and limbic inputs converge onto the same GC neurons and suggest BLA may shape cortical taste processing by dynamically gating the excitatory/inhibitory balance of the circuit.

Disclosures: **M.E. Stone:** None. **A. Maffei:** None. **A. Fontanini:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.15/O20

Topic: D.01. Chemical Senses

Support: NIDCD grant RO1-DC013770

Title: Amygdalo-cortical synaptic plasticity in primary gustatory cortex

Authors: *M. HALEY, A. FONTANINI, A. MAFFEI;
SUNY At Stony Brook, Stony Brook, NY

Abstract: Input from the basolateral amygdala (BLA) to primary gustatory cortex (GC) carries information on the hedonic value of a taste stimulus as well as anticipatory predictive cues. Previous studies have demonstrated that following taste learning, the functional connectivity between these two areas is increased. While this increase in connectivity suggests plastic changes

in the BLA-GC projection, details regarding the capacity for plasticity of this projection at the synaptic level remain unknown. Using whole-cell patch clamp recordings in GC slices combined with optogenetic activation of BLA terminal fields, we investigated how different physiological relevant patterns of activity promote plasticity induction at BLA-GC synapses. We developed two induction paradigms based on Hebbian learning rules, pairing presynaptic and postsynaptic activity, and varied the pattern of presynaptic activity. We found that phasic activation of BLA afferents (5ms pulses at 20 Hz) promotes long-term depression (LTD) of the synapse (% change: -24.6 ± 6.8, $p < 0.0001$, n = 10/14). Tonic activation of BLA afferents (6s ramp) promotes long-term potentiation (LTP) of the synapse (% change: 82.4 ± 19, $p < 0.0001$, n = 12/18). Our results indicate that BLA-GC synapses are capable of both LTD and LTP and that the pattern of presynaptic BLA activity dictates the direction of plasticity.

Disclosures: **M. Haley:** None. **A. Fontanini:** None. **A. Maffei:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.16/O21

Topic: D.01. Chemical Senses

Support: Consejo Nacional de Ciencia y Tecnologia of Mexico Grants 179484

Productos Medix ZIP0268

Title: Sucrose promotes quinine and capsaicin consumption in rats

Authors: *M. A. VILLAVICENCIO CAMARILLO[1], E. G. FONSECA DE LA CRUZ[2,1], S. A. SIMON[3], R. GUTIERREZ[1];
[1]Ctr. De Investigación Y Estudios Avanzados, Mexico, Mexico; [2]Inst. de Fisiología Celular, UNAM, México, Mexico; [3]Dept. of Neurobio., Duke Univ. Med. Ctr., Durham, NC

Abstract: Bitterness and pungency (mediated by the trigeminal system) in the oral cavity evolved to warn animals from consuming potential noxious substances. Nevertheless, learning and social induction, among other mechanisms, promote aversive chemical intake. For example, association between bitterness or burning substances with reinforcing tastes (e.g sweet or fat) are known to promote spicy food intake. We wondered whether a rewarding food (e.g. sucrose) is able to reinforce the consumption of aversive and/or burning stimuli (e.g. quinine hydrochloride [QHCl] or capsaicin, respectively). To address this issue, we trained one group of rats (Q; n=4) in a 30-minutes freely licking task to lick a spout at the central port of a behavioral box to obtain 10 µl drops of QHCl (0.3mM) and another group (CAP, n=4) to obtain 10 µl drops of capsaicin (4 ppm). Then, when a pause in licking greater than 1 s was detected, subjects were required to switch to a lateral port where they had to lick in order to obtain the either same number of drops

of sucrose (20%; 10 µl) or less if the rat made a pause of licking longer than 1 s. Therefore, in each trial, rats were forced to consumed aversive substances to get rewarding sucrose. We found that after 9 sessions of training rats stabilized the volume consumed of either QHCl (7.7 ml) or capsaicin (9.5 ml) to obtain a similar amount of sucrose (Q = 7.6 ml; CAP = 8.8 ml). Interestingly, in this sessions, Q group opted for drink smaller amounts of QHCl per trial compared with CAP group (11.8 vs. 20.6 drops). Nevertheless, sucrose obtained by Q rats was compensated by increasing the number of trials per session in comparison with CAP rats (Q = 68.35; CAP = 48.15). Then, to discard the possibility that rats drunk either QHCl or capsaicin because they were as rewarding as sucrose, we measured how much sucrose volume subjects will drink in the central port to obtain the same (or less) capsaicin or quinine volume in the lateral ports as a reward. We found that rats switched ingested a greater amount of sucrose (25 ml) than aversive stimuli (Q = 3.5 ml; CAP = 3 ml), suggesting that, in the first condition, aversive substances intake was only reinforced by sucrose. Together this preliminary data demonstrated that a rewarding taste promotes aversive or pungent stimuli consumption; as well, it is shown that rats maximize sucrose intake by using different strategies depending on the system that aversive substances are activating (bitter taste or burning sensation). In summary, these results highlight other mechanisms by which unpleasant substances consumption might be promoted.

Disclosures: **M.A. Villavicencio Camarillo:** None. **E.G. Fonseca de la Cruz:** None. **S.A. Simon:** None. **R. Gutierrez:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.17/O22

Topic: D.01. Chemical Senses

Support: CONACYT 179484

Productos Medix 0001275

Title: Orbitofrontal cortex neuron responses during a sweet intensity discrimination task in rats

Authors: *E. G. FONSECA DE LA CRUZ[1,2], A. MATSUMOTO TAKANE[3], M. VILLAVICENCIO[2], R. GUTIERREZ[2];
[1]Inst. De Fisiología Celular, UNAM, Mexico City, Mexico; [2]Pharmacol., CINVESTAV, Mexico City, Mexico; [3]Psychology, UNAM, Mexico City, Mexico

Abstract: Taste perception carries important information of food hedonic properties, thereby it has a key role in determining food consumption. It has been proposed that a distorted taste perception might underlie pathological eating patterns. Thereby it is important to develop

methodological ways to measure taste sensitivity and its underlying neural correlates. Orbitofrontal Cortex (OFC), also known as secondary gustatory cortex, might be involved in processing information about taste identification sensitivity. It has been shown that single neurons in OFC responds in an intensity dependent manner to sweet taste. Nevertheless, to our knowledge, OFC participation in processing sweet taste identification sensitivity has not been addressed. To addresss this issue, we trained rats in a sucrose discrimination paradigm that allows sucrose identification sensitivity measure by using psychophysics, and record single neuron activity in OFC while rats perform the task. Rats were trained to lick in a central spout to receive a single 15µl drop of 3% (low) or 18% (high) sucrose concentration and emit differential responses (left or right) according to the taste cue received. Correct responses were reinforced by three 15µl water drops. Additional Licks (AL's) after cue provides the reaction time to detect the sucrose drop. Once subjects learned the task, generalization sessions with intermixed training and probe trials were introduced. Probe trials (20% trials) consisted in the presentation of training and intermediate sucrose (3, 4.75, 7.5 or 11.75, 18%) that rats were required to classified as "low" or "high" concentration. This trials were unreinforced. High concentration response probability were obtained and fitted to a sigmoid function to obtain psychophysical sensitivity measurements. Preliminary results shows that subject reach ~>85% correct responses after ~20 training sessions. AL´s are significantly higher for high sucrose concentration, which might indicate palatability differences between training concentrations. AL´s for intermediate sucrose concentration were similar, nonetheless rats classified persistently intermediate low concentration (4.75%) as low and 11.75% as high, suggesting that rats were discriminating sucrose intensity per se and not AL's number. Preliminary data from 147 neurons are consistent with literature. We found neurons inhibited or excited by licking or cue, as well as neurons that fire synchronized with licking. Generalization sessions with electrophysiological recordings are about to be performed in order to evaluate OFC participation in sweet intensity perception.

Disclosures: **E.G. Fonseca de la Cruz:** None. **A. Matsumoto Takane:** None. **M. Villavicencio:** None. **R. Gutierrez:** A. Employment/Salary (full or part-time):; CINVESTAV. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CONACYT 179484, Productos Medix 0001275, ICYTDF-PICSA12-126, Salud2010-02-151001.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.18/O23

Topic: D.01. Chemical Senses

Support: Consejo Nacional de Ciencia y Tecnologia of Mexico Grants 179484

Productos Medix ZIP0268

Title: Optogenetic induced inactivation of the lateral orbitofrontal cortex during a sucrose freely licking task

Authors: *D. A. GARCÍA[1,2], L. PRADO[2], R. GUTIERREZ[2];
[1]INSTITUTO DE FISIOLOGIA CELULAR, UNAM, Mexico city, Mexico; [2]Pharmacol., CINVESTAV, Mexico City, Mexico

Abstract: The orbitofrontal cortex (OFC) is a brain structure that contains the secondary gustatory cortex, it is related to taste processing, but it is also involved in licking behavior, since direct infusion of a GABA A agonist (muscimol) into the lateral OFC fragment the microstructure of licking behavior (Gutiérrez, et al. J Neurophysiol, 95(1):119-133, 2006). However, these kind of pharmacological techniques have slow kinetics and poor reversibility. In this contextual frame, we decided to use an optogenetic approach and optrode recordings in the VGAT-ChR2-EYFP transgenic mice, which expresses channelrhodopsin in GABAergic interneurons that constitute approximately 20% of cortical neurons (Taniguchi et al. Neuron, 71: 995-1013, 2011). Briefly, the mouse was allowed to freely lick to obtain sucrose in a spout; each trial has three epochs:1) A baseline epoch, where the mouse had to lick an sipper with sucrose three times and at fourth lick, a LED stimulation was turned on. 2) In the stimulation epoch, mouse was stimulated with a power of 15 mW during 1s (10 ms pulse duration) at different randomly frequencies (4, 7, 14, 21, 30, 50 Hz) or during 1 s of constant stimulation. 3) A time out epoch, laser was turned off during the next second. We recorded activity of 110 neurons at the same time of task. We found a cortical modulation in firing rate during the stimulation epoch, were neuronal inhibition at high frequencies (30 and 50 Hz) and during 1s of continuous stimulation; this effect was reversible when the LED was turned off spiking activity rapidly returned to baseline levels. Behaviorally, we saw a slight alteration in rhythmicity of licking by the presentation of larger pauses (interlick intervals) in some animals (but not in all) at high frequencies and 1s continuous stimulation. Our data propose a viable model for study inhibitory cortical circuits at the subsecond level in sensory and cognitive functions without overtly affecting oromotor responses.

Disclosures: D.A. García: None. **L. Prado:** None. **R. Gutierrez:** None.

Poster

327. Taste System

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 327.19/O24

Topic: F.02. Animal Cognition and Behavior

Support: JSPS Grant B26290006

Title: Anandamide-induced network oscillation in the insular cortex implicated in taste-driven feeding

Authors: *Y. KANG;
Osaka Univ. Grad. Sch. Dent., Osaka, Japan

Abstract: Anandamide (AEA) and N-oleoylethanolamine (OEA) are produced or increased in the intestine and brain during fasting and satiety, respectively. Subsequently, the former facilitates food intake via activation of cannabinoid type 1 receptors (CB1Rs) while the latter decreases food intake via activation of peroxisome proliferator-activated receptor-α (PPAR-α) and/or G-protein-coupled receptor 119 (GPR119). Neuronal activity in the gastrointestinal region of the autonomic insula (GI-Au-I) that rostrally adjoins the gustatory insula (Gu-I) increases during fasting, causing appetite sensation while umami and sweet taste sensations in the Gu-I enhances appetite sensation in the GI-Au-I. Given that AEA induces neural coordination between the Gu-I and GI-Au-I, such coordination would be critically involved in inducing the taste-driven feeding. However, these possibilities have not been addressed. Here, we demonstrate with live imaging that application of AEA induces theta-rhythm oscillatory coordination between the Gu-I and GI-Au-I. This neural coordination was modulated by GABAB receptor-mediated feed-forward inhibition and was abolished by AM251, a CB1R antagonist and OEA, a GPR119 agonist and rolipram, a phosphodiesterase 4 inhibitor. We propose a novel brain mechanism in which taste-driven feeding is regulated by the neural coordination between the Gu-I and GI-Au-I through the opposing activities between the CB1R and GPR119. Our results provide a new insight into the higher-order brain mechanism responsible for emotional feeding behavior caused by taste recognition, in contrast to the known involvement of the hypothalamus in the regulation of food intake as nutrients.

Disclosures: Y. Kang: None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.01/O25

Topic: D.02. Auditory System

Support: NIDCD DC004274 (HvG)

Title: Exocytosis and energy metabolism from mitochondria at hair cell ribbon synapses

Authors: *K. LEAL[1,2], H. VON GERSDORFF[1,2];
[1]Oregon Hlth. and Sci. Univ., Portland, OR; [2]Vollum Inst., Portland, OR

Abstract: Hair cell afferent fiber synapses possess highly specialized active zones of glutamate release that enable rapid and faithful transfer on sensory information to neurons that innervate the brain. Hair cells are capable of releasing thousands of synaptic vesicles per second and continue to do so for tens of seconds unlike conventional synapses. However, the factors that contribute to the rapid supply of synaptic vesicles and sustained release at these ribbon-type synapses remain unknown. At presynaptic terminals, ATP consumption and production is highly regulated in response to synaptic activity. Given the high-activity levels of hair cells, mitochondria are strikingly abundant and are often located near to synaptic ribbons, suggesting a need for a large amount of local ATP production. Here, we investigated the contribution of ATP and mitochondrial respiration to synaptic transmission at the auditory hair cell to afferent fiber synapse of the adult bullfrog amphibian papilla. Using whole-cell paired recordings of a hair cell and afferent fiber terminal, we found that hair cell synapses maintain robust exocytosis in the absence of ATP provided by the patch pipette. This surprising result contrasts with results obtained at ribbon synapses in the retina. We examined exocytosis via simultaneous presynaptic capacitance changes and excitatory postsynaptic currents (EPSCs). Exocytosis runs down dramatically in the presence of ATP-γS, a non-hydrolyzable form of ATP. In hair cells, ATP-γS does not reduce calcium current amplitude, but does significantly decrease capacitance jumps with successive depolarizations of 200 ms, which deplete the readily releasable pool of vesicles. This suggests that an abundant supply of ATP is a necessary component for continuous vesicle release at hair cells synapses. To test the contribution of presynaptic mitochondrial ATP production to sustain synaptic transmission, we dialyzed hair cells with oligomycin, an inhibitor of mitochondrial ATP synthase, in the presence of ATP-γS to further out compete endogenous ATP. Inhibition of mitochondrial respiration in hair cells had effects similar to ATP-γS, greatly reducing exocytosis and EPSC amplitude. Our results indicate that hair cells maintain copious exocytosis by producing large amounts of ATP from mitochondria. We conclude that high levels of ATP are required for maintaining the readily releasable pool of synaptic vesicles during repeated stimulation of hair cell synapses.

Disclosures: **K. Leal:** None. **H. von Gersdorff:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.02/O26

Topic: D.02. Auditory System

Support: NIH grant DC013917 (OPG)

NIH grant DC04274 (HvG)

Title: Latency and efficiency of multivesicular release at hair cell ribbon synapses

Authors: *O. GROSS[1], H. VON GERSDORFF[2];
[2]Vollum Inst., [1]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Auditory hair cells encode features of acoustic stimuli in patterns of neurotransmitter exocytosis at ribbon-type synapses. This synaptic signal drives an excitatory postsynaptic current (EPSC) in afferent nerve fibers, which carry auditory information to the brain via spikes in the auditory nerve. Both rate and time codes of spikes are used to encode information. Spontaneous EPSCs observed in the afferent fiber when the hair cell is strongly hyperpolarized (V_M = -90 mV) exhibit a uniform time course and mean amplitude of ~60 pA. When hair cells are depolarized beyond the activation threshold of their L-type Ca^{2+} channels (about -65 mV), spontaneous EPSC amplitudes become more heterogeneous, exceeding 250-300 pA in some cases. Remarkably, the time course remains the same for all EPSC events, independent of amplitude. While recent work has shown that large EPSCs reduce the failure rate and improve phase-locking of afferent fiber spikes, the mechanisms and consequences of large EPSC events are not fully understood. To explore the mechanism of large events, we performed simultaneous whole cell recordings from hair cells and afferent fibers in the 400 Hz tonotopic region of the adult bullfrog amphibian papilla. Comparison of the hair cell membrane capacitance jump with the deconvolved EPSC produced by moderate hair cell depolarization suggests that large individual EPSCs arise from the simultaneous fusion of multiple synaptic vesicles at a single ribbon site (multivesicular release or MVR). To characterize MVR on a short time scale, we examined the latency of the first multiquantal event (MQE) during hair cell stimulation. We found that reliable low-latency (1-2 ms) MVR is achieved when ~4 Ca^{2+} channels are open per ribbon. The minimum Ca^{2+} current that reliably triggers low-latency MVR is only ~30% of the maximum Ca^{2+} current. Interestingly, this range of stimulation corresponds to the steepest region of the I-V curve of Ca^{2+} channels. Thus, MVR is maximally efficient over the same range of stimuli for which the Ca^{2+} current is best able to discriminate between stimulus strengths (i.e. the range where voltage sensitivity is maximal). Furthermore, a shift in the overall distribution of event amplitudes toward larger values was observed as the strength of the hair cell depolarization was increased. This result indicates that the mean event amplitude is modulated dynamically by stimulus strength. We suggest that this modulation has the potential to increase the efficiency of information coding at the first auditory synapse by supplementing the event rate code.

Disclosures: O. Gross: None. **H. von Gersdorff:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.03/O27

Topic: D.02. Auditory System

Title: Use of an *in vitro* screening platform to identify compounds that regenerate hair cells in the inner ear

Authors: *K. I. LORRAIN, A. DEARIE, M. POON, J. SEIDERS, J. ROPPE, P. PRASIT, D. LORRAIN;
Biol., Inception Sciences, Inc., San Diego, CA

Abstract: Loss of mechanosensory hairs cells in the inner ear is one of the main causes of sensorineural hearing loss. Although hair cell regeneration occurs in birds and amphibians, it does not occur in the adult mammal. Our goal here was to identify small molecule targets capable of inducing hair cell regeneration leading to hearing restoration. To this end we developed a high throughput assay for the detection of inner ear hair cells using the In Cell™ imaging system. Acute dissociation of the organ of Corti (oC) generates mouse otospheres which are capable of division and differentiation. Otospheres comprise a mixed population of cell types, including Sox2+ support and Myo7a+ hair cells. Cells are plated in a 96-well plate format, treated with compound and read on the In Cell™ imaging system. We have developed a series of algorithms using Developer™ software that accurately and quickly quantifies support cell proliferation and hair cell formation. Compounds showing activity were next validated in mouse oC explants. Follow up screens have been implemented to further validate each compound class.

Disclosures: K.I. Lorrain: None. **A. Dearie:** None. **M. Poon:** None. **J. Seiders:** None. **J. Roppe:** None. **P. Prasit:** None. **D. Lorrain:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.04/O28

Topic: D.02. Auditory System

Support: NOHR (2013-2014)

NIEHS P30 ES020957

Title: Inhibition of protein nitration attenuates cisplatin-induced modulation of LMO4 and mitigates the ototoxic effects

Authors: *S. JAMESDANIEL[1], R. RATHINAM[1], W. NEUMANN[2];
[1]Inst. of Envrn. Hlth. Sci., Wayne State Univ., Detroit, MI; [2]Pharmaceut. Sci., Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: Ototoxicity is one of the major side-effects of the commonly used chemotherapeutic agent cisplatin. More than 50% of patients treated with this anti-cancer drug develop hearing loss and the incidence of ototoxicity increases significantly in children, particularly, at higher doses

of cisplatin with debilitating consequences. Therefore, it is extremely important to delineate the signaling pathways that facilitate ototoxicity in order to design rational therapeutic strategies to prevent cisplatin-induced hearing loss and preserve the quality of life in cancer survivors. During the past few years, a good deal of progress has been made in elucidating the mechanisms underlying cisplatin-induced ototoxicity. Nevertheless, the attenuation of this side-effect with otoprotective compounds is still inadequate. We reported that cisplatin treatment leads to the nitration of cochlear LMO4 and hypothesized that LMO4 nitration plays a pivotal role in facilitating cisplatin-mediated ototoxicity. In this study we provide evidence that inhibition of protein nitration by SRI110, a peroxynitrite decomposition catalyst, not only attenuates cisplatin-induced modulation of LMO4, but mitigates the ototoxic side-effects of cisplatin, in organ of Corti cell cultures. SRI110 is a selective inhibitor of protein nitration that spares the superoxide radical. Treatment of UBOC1 cells, which has been used as a model in ototoxicity studies, with cisplatin (10 μM) for 24 hours, induced apoptosis, as indicated by an increase in the expression of active caspase 3, and decreased LMO4 levels, as indicated by immunoblotting and immunostaining with anti-LMO4. Co-treatment with SRI110 (50 μM) attenuated the cisplatin-induced decrease in LMO4. Moreover, it significantly reversed the cisplatin-induced cellular apoptosis ($p<0.5$), which was quantified by flow cytometry analysis of active caspase 3 expression. The modulation of LMO4 levels by cisplatin and SRI110 treatment correlated with the changes in the active caspase 3 levels ($r = -0.891$) indicating the functional significance of LMO4 nitration in cisplatin-induced ototoxicity and the otoprotective efficacy of SRI110. Delineation of this key signaling mechanism provides a basis for identifying potential interventional targets, and in addition, provides information vital to the search for better otoprotective compounds. This study was supported by NOHR grant (2013-2014) and NIEHS P30 Grant (P30 ES020957).

Disclosures: **S. Jamesdaniel:** None. **R. Rathinam:** None. **W. Neumann:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.05/O29

Topic: D.02. Auditory System

Support: Amelia Peabody Charitable Fund

NIH Grant P30 DC05029

NIH Grant R01DC012838-01A1

Title: Damage to sensory hair cells occurs via excessive activation of ionotropic glutamate receptors that is independent of afferent and efferent innervation

Authors: *L. SHEETS;
Mass Eye & Ear Infirmary/ Harvard Med. Sch., Boston, MA

Abstract: Excessive release of glutamate from sensory hair cells plays a central role in eliciting the pathological events that follow damaging sound exposures. The effects of excess glutamate on afferent fibers innervating hair cells has been well characterized—perfusion of AMPA/kainate type GLUR agonists into mammalian cochlea has been shown to induce pathological dendritic swellings similar to those observed in noise-exposed ears. But less is known about the cellular mechanisms instigating noise-induced hair-cell damage, and whether glutamate excitotoxicity also contributes to pathological changes in hair cells has not been directly examined. To address whether glutamate excitotoxicity damages sensory hair cells, I examined lateral-line neuromasts (NMs) in 5-day-old zebrafish larvae exposed to drugs that mimic glutamate-induced excitotoxic trauma. Exposure to kainic acid (KA) resulted in profound swelling of lateral-line afferent terminals analogous to that observed in KA exposed mammalian cochleae. Unexpectedly, KA exposure also contributed to hair cell damage and significant hair cell loss. To test whether hair-cell damage was a secondary effect from over stimulating innervating afferent neurons, I exposed *neurog1a* morphants—fish that have morphologically mature and electrically active NM hair cells that are devoid of afferent and efferent innervation—to KA. Significant hair-cell loss occurred in KA exposed *neurog1a* morphants, and the loss was comparable to that observed in wild-type siblings. The action of KA is likely specific, as NM hair cell loss occurred in a dose-dependent manner and was prevented when morphants were co-exposed to the competitive AMPA/kainate receptor antagonist CNQX. In addition, transient increases in intracellular calcium were observed in morphant NM hair cells following KA exposure, frequently preceding hair-cell death. Cumulatively, these data indicate that excessive glutamate signaling mediates damage to sensory hair cells independent of damage to postsynaptic terminals, and suggest that excess glutamate resulting from noise exposure directly contributes to noise-induced hair-cell damage and death.

Disclosures: L. Sheets: None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.06/O30

Topic: D.02. Auditory System

Support: NIH Grant DC013304

Title: Targeted deletion of oncomodulin leads to changes in auditory thresholds independent of outer hair cell loss

Authors: *D. D. SIMMONS, A. AZGHADI, M. KAZANTSEV, A. J. HORNAK;
Integrative Biol. and Physiol., UCLA, Los Angeles, CA

Abstract: The regulation and control of Ca^{2+} is one of the major challenges of cochlear hair cells. Oncomodulin (Ocm) is an EF-hand Ca^{2+} binding protein, a member of the parvalbumin protein family, and found almost exclusively in the outer hair cells (OHCs). In the cochlea, OHCs use a prestin-based electromotility mechanism to amplify vibrations of the cochlear partition that directly enhance sensitivity and frequency selectivity, and serve as the primary targets of a brainstem efferent pathway that may protect the ear from noise damage. Cochlear OHCs are one of the most prominent targets of noise and aging defects. We hypothesized that deletion of a major Ca^{2+} buffer in OHCs would lead to hearing loss. Using a targeted deletion of Ocm, we investigated the relationship between OHC loss, hearing thresholds, and the presence of efferent terminals. Targeted deletion of Ocm resulted in progressive hearing loss beginning in high frequencies around 3 months with significantly elevated threshold shifts. However at 3 months, there was typically little evidence of OHC loss in Ocm mutants. Around 4 months, Ocm null mutants demonstrated increasing amounts of hair cell loss in basal high frequency regions. We also found significant changes in prestin-immunoreactivity occurred around 4 months. Mutant animals exhibited a basal to apical prestin-immunoreactive intensity gradient. Additionally in mutants, OHCs were shorter especially in apical regions. In 3-month-old mutants, efferent terminals were found on OHCs and appeared normal. However after 4 months, Ocm mutant mice had basal (high frequency) regions where OHCs lacked efferent terminals. Efferent terminals showed significant remodeling in areas of the cochlea where OHCs were absent. The loss of efferent terminals coincided with changes in prestin expression. These data suggest that targeted deletion of Ocm leads to progressive elevation of hearing thresholds that are independent of OHC loss. Changes in prestin expression and efferent innervation seem to occur prior to OHC loss.

Disclosures: D.D. Simmons: None. **A. Azghadi:** None. **M. Kazantsev:** None. **A.J. Hornak:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.07/O31

Topic: D.02. Auditory System

Title: The effects of the somatostatin analogue pasireotide and role of NFAT in protection of auditory hair cells after aminoglycoside exposure

Authors: *V. RADOJEVIC, A. PERKOVIC, D. BODMER;
Dept. of Biomedicine, Univ. Hospital, Clin. For Otorhinolaryngolog, Basel, Switzerland

Abstract: Hearing impairment is a global health problem with high socioeconomic impact. Damage to auditory hair cells in the inner ear due to aging, disease, acoustic trauma, or exposure to ototoxins underlies most cases of sensorineural hearing loss. Since the mammalian ear cannot replace damaged hair cells, loss of hearing is irreversible and progresses throughout life. We previously demonstrated that the Ca2+ -sensitive neuropeptide somatostatin and its analogue octreotide can protect hair cells from gentamicin-induced hair cell death *in vitro*, and that SST receptors are expressed in the mammalian inner ear. Aminoglycosides trigger an influx of calcium ions (Ca2+) and rapid rise in intracellular calcium in hair cells which activates intracellular signaling cascades that may terminate in both survival or apoptotic events. Somatostatin (SST), acts via a family of G-protein-coupled receptors (SSTR1-SSTR5) that are differentially distributed throughout the central nervous system. SST receptors provide neuroprotection by direct coupling to voltage-dependent Ca2+ channels which are inhibited by SST binding to its receptors. In the present study, we report that the SST analogue pasireotide (high affinity to SST receptor subtypes SSTR1, 2, 3 & 5 with improved half-life vs. octreotide) also prevents gentamicin-induced hair cell death. We explored whether or not NFAT, a Ca2+ -sensitive transcription factor, may play a role in SST-mediated signaling. We found that activated NFAT translocated to the nuclear fraction of organ of Corti explants exposed to gentamicin, and this was prevented by pasireotide. To determine whether or not NFAT mediated the protection of hair cells by pasireotide, we treated OC's with the direct NFAT inhibitor VIVIT-11. Indeed, VIVIT-11 was able to significantly protect hair cells from gentamicin-induced apoptosis and, similar to the effects of pasireotide, prevented the gentamicin-dependent nuclear translocation of NFAT. Finally, we measured expression of NMDA1 receptor and PI3K, genes previously shown to be related to gentamicin induced HC death. Both pasireotide and VIVIT-11 significantly prevented the gentamicin-induced change in expression of the NMDA receptor and PI3K. These data suggest a model in which somatostatin analogues antagonize aminoglycoside induced alterations in cellular Ca2+ fluxes, leading to preservation of hair cell survival pathways (PI3K, NMDA) in a manner requiring NFAT. Our data suggest that SST analogues and NFAT inhibitors may offer new therapeutic possibilities for treatment of hearing loss.

Disclosures: **V. Radojevic:** None. **A. Perkovic:** None. **D. Bodmer:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.08/O32

Topic: D.02. Auditory System

Support: Indian Council of Medical Research (ICMR) SRF Project

All India Institute of Medical Sciences New Delhi. Intramural Project A-218.

Title: A stereological investigation of the parvalbumin positive neurons in the adult human spiral ganglion

Authors: C. KAUR[1], T. C. NAG[1], A. THAKAR[2], D. N. BHARDWAJ[3], T. G. JACOB[3], *T. ROY[4];
[1]Anat., [2]Otorhinolaryngology, [3]Forensic Med. and Toxicology, [4]All India Inst. Med. Sci., New Delhi, India

Abstract: It is well established that different Ca binding proteins have distinct buffering properties and are related to neuronal firing rates. Parvalbumin (PV) is associated with highly metabolically active neurons in comparison to other calcium binding proteins. The expression of PV has been used to describe anatomically various sensory pathways, including the spiral ganglion (SG). To complement our previous findings, where the degree of the intensity of immunoreactivity of PV in the SG were 'weakly' and 'strongly' positive, in the present study we attempted to quantify and compare the PV and cresyl violet (CV) positive neurons, separately using unbiased stereology to estimate total number, mean volume of neurons and the volume of SG in the adult human. Estimation of total number of neurons in the SG at various ages and their functional status is important as these neurons are constantly exposed to noise and other environmental factors leading to excess of neurotransmitter release that may result in loss of SG neurons. Five adult human cadaveric heads were obtained from the forensic science mortuary at All India Institute of Medical Sciences, New Delhi, with approval from institute ethics committee. These were accident victims (from the third decade of life) and had no history of inner ear disease or hearing loss before death. The temporal bone containing the SG was dissected, fixed, decalcified, cryoprotected and serially sectioned (30μm) in the coronal plane. Every 7th section was immunostained with PV (Abcam, ab11427, 1:5000) and with CV separately, using standard protocol. PV and CV stained sections were used independently for estimation of the total number of neurons (Optical Fractionator), neuronal volume (Cavalieri) and the volume of its nucleus (Nucleator) with StereoInvestigator software (Microbrightfield Inc. VT, USA). The estimated total number of SG neurons was 27,723 ± 2371.03 and 27,484 ± 3251.3 respectively in the PV and CV stained sections. There was no significant difference in the counts from CV stained sections when compared with PV immunostained sections (Student's t test, $p = 0.911$). This count of neurons is nearly half of what has been previously reported for human SG by other groups. The mean volume of SG neurons and its nucleus was 130.57 um3 ± 37.16 and 131.68 um3 ± 50.68 um3; and 1.8 um3 ± 0.35 and 2.12 um3 ± 0.35. The mean volume of SG was 3569.45 um3 ± 791.22 and 3487.63um3 ± 951.49 in the PV and CV stained sections, respectively. Since both methods to estimate neuronal numbers yielded similar results, therefore we conclude that there is preponderance of PV positive neurons in the human SG, implying its predominant role in Ca buffering.

Disclosures: C. Kaur: None. **T.C. Nag:** None. **A. Thakar:** None. **D.N. Bhardwaj:** None. **T.G. Jacob:** None. **T. Roy:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.09/O33

Topic: D.02. Auditory System

Title: Effective protection against severe noise-induced hearing loss by a small molecule clinical drug candidate following daily, post-trauma systemic administration

Authors: *J. DYHRFJELD-JOHNSEN, M. PETREMANN, V. BRIEUC, A. BROUSSY; Sensorion, Montpellier, France

Abstract: Sensorineural hearing loss is caused by damage to the sensory hair cells and neurons of the cochlea and is the most common type of permanent hearing loss (American Speech-Language-Hearing Association; ASHA). Among adults, the 2 main causes of sensorineural hearing loss are excessive noise exposure and aging (Hearing Loss Association of America; HLAA). Currently no approved pharmaceutical treatment exists and recent meta-analysis of the standard-of-care, off-label use of corticosteroid therapy have concluded that neither systemic nor intratympanic administration has any significant treatment effect (Crane et al. 2015, Laryngoscope 125(1):209-17). We here demonstrate that the small molecule, clinical drug candidate SENS-90 significantly reduces permanent hearing loss and loss of outer sensory hair cells in a rat model of severe noise induced hearing loss after daily, post-trauma, systemic administration. Following baseline audiometry, 7 week old awake and behaving male Wistar rats were exposed to 120 dB octave band noise (8-16 kHz) for 2 hours on a slowly rotating platform in a sound-attenuating cubicle. SENS-90 (n=7) or placebo (n=7) treatment was initiated after the end of acoustic trauma exposure using intraperitoneal administration and continued daily until day 13. Both SENS-90 and placebo treated animals displayed up to ~60 dB temporary ABR threshold shifts at 24h (8/16/24 kHz) accompanied by strong or complete suppression of DPOAE amplitudes (4/8/16/24/32 kHz). However, on day 14, SENS-90 treated animals displayed up to ~60% lower permanent ABR threshold shifts and up to ~60% higher DPOAE amplitudes. Taking into account potential variability of individual acoustic trauma, both recovery of ABR thresholds and DPOAE amplitudes from 24h to day 14 were also determined to be significantly improved after SENS-90 treatment. The functional audiometry data were supported by significantly reduced mean outer hair cell loss after drug treatment determined from cochleograms constructed from cell counts in fixed cochlea at day 14. Altogether, these results demonstrate that daily, systemic administration of the small molecule clinical candidate drug SENS-90 initiated after severe acoustic overexposure strongly and significantly protects against permanent hearing loss and cochlear cell loss.

Disclosures: J. Dyhrfjeld-Johnsen: A. Employment/Salary (full or part-time):; Sensorion. **M. Petremann:** A. Employment/Salary (full or part-time):; Sensorion. **V. Brieuc:** A. Employment/Salary (full or part-time):; Sensorion. **A. Broussy:** A. Employment/Salary (full or part-time):; Sensorion.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.10/O34

Topic: D.02. Auditory System

Support: Fondi di Ateneo 2014 - UCSC - Italy

Title: The upregulation of Nrf2/HO-1 pathway restores cochlear redox homeostasis after noise exposure

Authors: *D. TROIANI[1], F. PACIELLO[2], R. ROLESI[2], S. L. M. ERAMO[1], A. R. FETONI[2]; [1]Inst. Physiol. Univ. Cattolica Sch. of Med., Rome, Italy; [2]Otolaryngology, Univ. Cattolica Sch. of Med., Rome, Italy

Abstract: The redox-sensitive transcription nuclear erythroid 2-related factor 2 (Nrf2) plays a critical role in the maintenance of cellular homeostasis under stress conditions by regulating endogenous cellular defenses against oxidative stress, including heme-oxygenase 1 (HO-1) activation. The progressive increase of reactive oxygen species (ROS) in conjunction with an imbalance of antioxidant defenses, have been demonstrated to play a significant role in Noise-induced hearing loss (NIHL). Namely, in this study we assessed the efficacy of a polyphenol compound, Rosmarinic Acid (RA), to activate Nrf2/HO-1 pathway, by potentiating the endogenous antioxidant defenses in a model of NIHL. Wistar rats were exposed to a pure tone (120 dB, 10 kHz) for 60 min in an anechoic room. One hour before and for the consequent 3 days, animals were treated with RA (10 mg/kg bw i.p.). Cochlear injury was evaluated by assessing functional (ABR recording) and morphological alterations (OHC survival). Oxidative stress was estimated by immunostaining or Western blot procedures to detect superoxide amount (DHE assay), lipid peroxidation (4-HNE) and the level of endogenous antioxidant responses (superoxide dismutase 1 and 2 and Nrf2-HO-1 pathway). Noise exposure induces oxidative stress in the cochlea, as indicated by superoxide and lipid peroxidation over-expression. To face the oxidative stress, the endogenous defense system is as well activated, as shown by the slight expression of SOD1 and SOD2. In addition, we observed the activation of the Nrf2/HO-1 pathway after noise exposure. However, the endogenous antioxidant system failed to restore the redox homeostasis and its activity was unable to prevent the cochlear damage. On the contrary, Nrf2/HO-1 signaling pathway was potentiated after RA administration, as well as SODs activity. This enhancement of endogenous antioxidant defenses was responsible for the reduction of oxidative stress parameters. In conclusion, the induction/activation of Nrf2-ARE signaling pathway and the up-regulation of endogenous antioxidant responses represent a relevant molecular mechanism to restore the redox imbalance caused by noise overexposure.

Disclosures: **D. Troiani:** None. **F. Paciello:** None. **R. Rolesi:** None. **S.L.M. Eramo:** None. **A.R. Fetoni:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.11/O35

Topic: D.02. Auditory System

Support: The Knowles Hearing Center, Northwestern University

Title: Differential influences of visual-task performance on cochlear responses in musicians and non-musicians

Authors: ***S. BOOTHALINGAM**[1], M. HALINSKI[1], C. E. MURRAY[1], J. LEE[2], B. A. WRIGHT[1], S. DHAR[1];
[1]Northwestern Univ., Evanston, IL; [2]Univ. of Wisconsin, Madison, WI

Abstract: Introduction: The corticofugal neural network from the auditory cortex influences the functioning of sub-cortical systems through auditory and cross-modal attention. Activation of this network selectively adjusts gain in the system, improving the signal-to-noise ratio of the incoming sensory stream. The final leg of this network is the medial olivocochlear system (MOC) in the brainstem, which directly inhibits cochlear activity. The strength of this cochlear inhibition appears to be greater in musicians than non-musicians when induced using only auditory stimuli. Here we asked whether cochlear responses were also differentially affected in these two populations during performance of a demanding visual task. Method: Inhibitory activity of the MOC was monitored using distortion product otoacoustic emissions (DPOAEs), sounds generated by the cochlea in response to a pure-tone pair. DPOAE levels were measured in the right ear of participants. Musicians had received formal musical training, and currently practiced music >3 hours/week. Non-musicians had not received formal musical training or played music regularly in the last 4 years. The demanding visual task was Rapid Serial Visual Presentation (RSVP), in which images were presented in rapid succession and participants were required to respond upon detecting a repeated image. DPOAEs were measured in three conditions: with no visual task (baseline), while performing the visual task (RSVP), and immediately after performing the visual task (afterRSVP). DPOAE level differences between the baseline and the RSVP condition provided a metric of visual-task mediated changes in the cochlea, while those between the baseline and the afterRSVP condition quantified the decay of the influence of the prior visual task. Results: In preliminary data, DPOAE levels were significantly lower in musicians (n=4) than non-musicians (n=5) across all three conditions. Performing the RSVP task reduced the DPOAE level for both groups (re: each group's baseline), but the reduction was significantly smaller in musicians compared to non-musicians. The

influence of the visual task did not persist in the afterRSVP condition for either group. Conclusion: The smaller influence of the visual task, combined with the overall lower DPOAE levels, in musicians than non-musicians, raises the possibility that efferent inhibition at the cochlea in musicians may be at a constant maximum. This state could result from the extended auditory-visual training in musicians. In contrast, in non-musicians, the tonic level of cochlear efferent inhibition may be low and increase only as required by task demands.

Disclosures: S. Boothalingam: None. **M. Halinski:** None. **C.E. Murray:** None. **J. Lee:** None. **B.A. Wright:** None. **S. Dhar:** None.

Poster

328. Auditory Processing: Mechanoreceptors and Cochlea

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 328.12/O36

Topic: D.02. Auditory System

Support: Fonds de recherche en santé du Québec

Natural Sciences and Engineering Research Council of Canada

Title: The use of electrocochleography in the diagnosis endolymphatic hydrops without vertigo

Authors: *M. MAHEU[1,3], S. ALHABIB[2], F. CHAMPOUX[1,3], I. SALIBA[2];
[1]Univ. of Montreal, Montreal, QC, Canada; [2]Dept. of Surgery, Div. of otorhinolaryngology-Head & Neck Surgery, Univ. of Montreal, University of Montreal, QC, Canada; [3]Ctr. for Interdisciplinary Res. in Rehabil. of Greater Montreal, Raymond-Dewar Inst., Montreal, QC, Canada

Abstract: Introduction Electrocochleography has been extensively studied in the diagnosis of Meniere disease (MD). The different categories of MD are well described by the American Academy of Otolaryngology- Head and neck surgery (AAO-HNS). The main criteria are vertigo, ear fullness, tinnitus and fluctuating sensorineural hearing loss. All the categories of MD described by AAO-HNS have a common symptom: vertigo. Although, many patients report ear fullness without vertigo, not caused by external or middle ear pathology, and therefore are often misdiagnosed. Electrocochleography (ECochG), using SP/AP amplitude ratio and SP/AP area ratio, has been demonstrated as a specific and sensible objective tool in the diagnosis of endolymphatic hydrops in MD. Objective and hypothesis The main objective of the study is to use ECochG to differentiate subjects that report ear fullness without vertigo, compared to controls. We believe that the use of SP/AP area ratio will allow differentiating between controls and the ear fullness group. Methodology 40 patients were recruited for the present study (20 controls and 20 with ear fullness). We recorded ECochG using an extra-tympanic electrode in each of these patients and studied the SP/AP amplitude ratio and the SP/AP area ratio. We then

compared the results from the ear fullness group to the results from the control group. Results There was a statistically significant difference (p=0.019) for SP/AP area ratio between the control group (Mean=1.48 SD=0.29) and ear fullness group (Mean=6.03; SD=7.16). However, no significant difference (p=0,063) could be observed for the SP/AP amplitude ratio between the control group (M=19,55; SD=9,74) and ear fullness group (M=30,06; SD=20,31). Conclusion It is shown that SP/AP area ratio can differentiate between patients having endolymphatic hydrops symptoms and controls. This is relevant because we can now identify objectively, using the SP/AP area ratio, the source of the symptoms expressed by the patient and possibly treat the condition more efficiently.

Disclosures: **M. Maheu:** None. **S. Alhabib:** None. **F. Champoux:** None. **I. Saliba:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.01/O37

Topic: D.03. Multisensory Systems

Title: Anatomical similarity is mandatory to provide body ownership toward body-shadow

Authors: *A. KANAZAWA, K. KODAKA;
Grad. Sch. of Design & Archtecture, Nagoya Ci, Nagoya-Shi, Japan

Abstract: Rubber hand illusion (RHI) can provide body ownership (BO) to a fake hand based on spatio-temporal correlation between visual and tactile stimulations. Another type of RHI that has gained increasing interest over the last few years is where we see a rubber hand (on desk) moving in sync with the movement of a hand (under the desk). Researchers have controlled an artificial hand (such as a robot hand) in sync with the hand movement, completing the vision-proprioception correlation (Casper et al., 2014). On second thought, the use of a body-shadow seems a quick and natural way to realize such a correlation; however, no research has directly focused on them, despite there being an increasing number of studies showing that the visualization of the body-shadow can change spatial perception (Kurlen et al., 2014). We aim at verifying an effect of body-shadow on RHI with a proprioceptive drift (PD) distance as an indicator of BO. We set three acrylic plates vertically at a suitable interval, where we can see a shadow of a right hand (on the middle) and a rectangle cloth (on the middle or the bottom) on screen of the top plate via a light source installed on the floor. Three shadow environments are designed in the following: the first is hand shadow (HS), where the top panel shows the right hand's shadow in a straightforward manner, the second is rectangle shadow (RS), showing the shadow of the rectangle cloth (clinging to the right hand) that moves in sync with the hand movement, and the third is unmoved rectangle shadow (URS), showing the shadow of the rectangle cloth (on the bottom) that remains stable regardless of the hand movement. In each

environment, 12 participants looked at the shadow for 1 min while not moving the hand (Unmoved) and moving the hand back and forth (Moved), in order. After each task, they were asked to indicate where they felt their right hand was located by the left hand with eyes closed (PD measurement) and to answer some simple questions. The result showed that RS and URS did not produce PD at all, regardless of whether there is hand movement; while, HS involved a significant PD compared with RS and URS, where PD magnitude was significantly larger in Moved (4.2 cm on average) than in Unmoved (2.0 cm on average). The questionnaire result also showed that the subjective strength of the BO was significantly larger in HS, while there was no main effect of the hand movement in HS. These indicate that anatomical similarity to body-part is mandatory to provide BO toward the body-shadow. Interestingly, shifting from Unmoved to Moved condition improved only the PD's magnitude; it did not improve the subjective strength of BO. The observed discrepancy is discussed considering the effect of time.

Disclosures: A. Kanazawa: None. **K. Kodaka:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.02/O38

Topic: D.03. Multisensory Systems

Support: NIH/ NICHD grant 1R01HD071978-01A1

Title: Axonal density in the ipsilesional and contralesional corticospinal tracts post stroke and relationship to corpus callosum microstructure

Authors: M. LAZAR[1], A. GEORGE[1], Y. LUI[1], V. ALURU[1], S. BILALOGLU[1], D. GELLER[1], *P. RAGHAVAN[2,1];
[1]New York Univ. Sch. of Med., New York, NY; [2]Rehabil. Med., New York Univ. Langone Med. Ctr., New York, NY

Abstract: A stroke can lead to persistent upper limb impairment on one side of the body. Increased activation of the undamaged contralesional motor areas has been shown to correlate with better motor recovery at a later time. This suggests that contralesional neural substrates can potentially drive motor output on the affected side of the body. The corpus callosum synchronizes large scale networks across the two hemispheres and may play a key role in transfer of function between the two sides. The purpose of this study was to examine the interaction between the ispilesional and contralesional corticospinal tracts (CST) and the corpus callosum (CC) as substrates of impaired hand function and of function transfer between the two hands. Thirteen patients with unilateral hand motor deficits at least 4 months post stroke participated in the study. Diffusion tensor imaging (DTI) was employed to derive Fractional

Anisotropy (*FA*). Microstructural white matter properties were also described using Diffusional Kurtosis Imaging (DKI), which include 1) Axonal Water Fraction (f_{axon}), a metric reflective of axonal density and caliber, 2) Intra-axonal Diffusivity (D_{axon}), which describes the overall organization of the intra-axonal milieu, and 3) Axial (AD_{extra}) and 4) Radial (RD_{extra}) extra-axonal diffusivities, which reflect the organization of the glia and the extracellular space. Mean *FA* and DKI metrics were obtained for the CSTs and for the posterior body of the CC, which connects the motor and somatosensory cortices of the two sides. Extent of arm motor impairment was measured using the Fugl-Meyer Scale (FMS). We found that the *FA* and f_{axon} of the ipsilesional CST were significantly decreased compared to that of the contralesional CST ($p<0.005$). Moreover, both the FA and f_{axon} of the ipsilesional CST were significantly correlated with arm motor impairment as measured by the FMS ($r/FA=0.611^*$; $r/f_{axon}=0.561^*$; $^*p<0.05$). No correlations were found between the FMS and the diffusion metrics of the contralesional CST ($p>0.05$). FA & f_{axon} of the ipsilesional CST correlated with the FA of the contralesional CST and with the FA & f_{axon} of the corpus callosum ($r>0.5$, $p<0.05$). These results indicate that functional hand motor impairment relates to microstructural deficits of the affected CST, with axonal loss due to Wallerian degeneration likely the primary driver of this relationship. CST relationships with CC support the hypothesis of inter-hemispheric transfer of information as a potential remediation strategy.

Disclosures: **M. Lazar:** None. **A. George:** None. **Y. Lui:** None. **V. Aluru:** None. **S. Bilaloglu:** None. **D. Geller:** None. **P. Raghavan:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.03/O39

Topic: D.03. Multisensory Systems

Support: NIH R01-EY020484

Title: Neurodynamics of letter perception in blind and sighted readers

Authors: ***S. TENG**[1], R. CICHY[1], D. PANTAZIS[2], V. SOMMER[1,3], A. OLIVA[1]; [1]CSAIL, [2]McGovern Inst. for Brain Res., MIT, Cambridge, MA; [3]Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: A large part of cortex in humans is dedicated to visual processing. However, in blind individuals visual cortex does not fall silent, but rather responds to a wide range of nonvisual tasks. What is the role, then, of so-called visual cortex and representations it mediates in blind persons, and how does it compare to representations in the sighted? To address these questions, here we used magnetoencephalography (MEG), multivariate pattern analysis, and

representational similarity analysis (RSA) to compare letter recognition in visual, auditory, and tactile modalities. We presented blind and sighted volunteers with 10 single letters in random order while recording brain activity. In separate experiments, sighted subjects (N=13) were presented with lowercase Roman visual letters, blind subjects (N=11) were presented with Braille tactile letters, and both groups (N=7 blind and sighted) were presented with spoken auditory letters. We compared the representation of letters across blind and sighted subjects as well as modalities using RSA. We found high representational similarity for tactile letters at ~200 ms and visual letter representations at ~600 ms, indicating a possible common processing mechanism. This result is not likely due to sub voce vocalization: representational similarity between spoken and tactile representations in the blind were more diffuse and peaked at later times. The results suggest that brain regions recruited crossmodally may be performing some common underlying computations for analogous tasks, but that letter reading is overall largely driven by distinct processes across sensory modalities and blind vs. sighted populations. This work was supported by NIH R01-EY020484 to A.O. and conducted at the Athinoula A. Martinos Imaging Center at MIT's McGovern Institute for Brain Research.

Disclosures: **S. Teng:** None. **R. Cichy:** None. **D. Pantazis:** None. **V. Sommer:** None. **A. Oliva:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.04/O40

Topic: D.03. Multisensory Systems

Support: European Research Council (EU-ERC 283567)

Netherlands Organisation for Scientific Research (NWO-VICI: 453-11-001; NWO-VENI: 451-10-017)

Title: Causal inference in self-motion estimation

Authors: ***A. TER HORST**, M. KOPPEN, L. P. J. SELEN, W. P. MEDENDORP; Radboud Univ. Nijmegen, Donders Ctr. For Cognition, Nijmegen, Netherlands

Abstract: While in motion humans face a vast amount of, often ambiguous, sensory cues. An observer might use these cues to infer the amount of self-motion, but only after having inferred whether these cues originate from one or multiple sources. During passive self-motion, the visual and vestibular cues are the most salient cues for estimating self- and object-motion. It is known that if both cues are integrated the brain follows the rules of Bayesian optimal integration, however it is unknown under what circumstances the brain associates both cues as coming from a single source and when the brain segregates both cues for separate processing. In other words,

when will a vestibular based estimate of self-motion be pulled towards a discrepant visual cue [that represents object-motion] and when will the brain decide that the cues have different causes and should be segregated? In this study we assess this causal inference problem on the integration and segregation of visual and vestibular cues during passive self-motion using Bayesian inference of a structural causality model. Participants were seated on a linear sled embedded in a virtual reality environment. They were subjected to linear motion that elicited both vestibular and visual cues. A discrepancy in motion amplitude between the visual and vestibular cues was added during these motions. Participants performed a two-alternative forced-choice task, indicating which of two sequential displacements was largest. Results indicate that observers start to segregate cue information when the discrepancy increases. The point on which segregation starts (occurs) depends on variability and can be modeled using a hierarchical probabilistic mixture of integration and segregation based responses.

Disclosures: **A. Ter Horst:** None. **M. Koppen:** None. **L.P.J. Selen:** None. **W.P. Medendorp:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.05/O41

Topic: D.03. Multisensory Systems

Support: NIH/ NICHD Grant 1R01HD071978-01A1

Title: Eye-hand coordination for adaptation of hand posture to object shape

Authors: ***A. YOUSEFI**, S. BILALOGLU, P. THAI, V. ALURU, Y. LU, J.-R. RIZZO, P. RAGHAVAN;
Rehabil. Med., New York Univ. Sch. of Med., New York, NY

Abstract: We grasp objects of various shapes during daily activities. Visual information about the perceived shape of the object can inform planning of hand posture for efficient grasp. A clear understanding of the mechanisms of eye-hand coordination and adaptation of hand posture to object shape is necessary to develop appropriate treatment strategies for restoration of coordination and dexterity in patients with impaired hand function such as after a stroke. The purpose of this study was to examine the relationship between gaze location and hand posture during reaching and grasping objects of different shapes in healthy subjects. 10 neurologically intact, right-hand dominant subjects participated in the study. The subjects were asked to sit at a table with the grasping hand on the table in front of them with the fist closed. The subjects were asked to reach and grasp the object positioned at 75% of arm's length in front of them, and repeat the task 7 times for 9 different objects with unique shapes (requiring differential angular

excursions of 5-10 degrees at the PIP/MCP joints). The focal point of binocular gaze was tracked by Eyelink2 eye tracker (SR Research Ltd, Ottawa, Ontario, Canada), and the location of the subject's head, hand, forearm, arm and shoulder were tracked by motion sensors attached to the limb segments and to the object (6DOF Ascension sensors, Ascension Technology Corporation Shelburne, Vermont). Adaptation of hand posture to object shape was measured using Cyberglove. (Cyberglove system, San Jose, USA). We examined the position of the focal point of gaze during the various phases of reach-to-grasp: prior to reach onset, and during reach acceleration, deceleration and grasp. The results suggest that examination of gaze patterns can inform planning strategies for control of hand shape during grasping. We can use this information as a benchmark to assess deficits in eye-hand coordination during reach-to-grasp in patients with stroke.

Disclosures: **A. Yousefi:** None. **S. Bilaloglu:** None. **P. Thai:** None. **V. Aluru:** None. **Y. Lu:** None. **J. Rizzo:** None. **P. Raghavan:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.06/O42

Topic: D.03. Multisensory Systems

Support: ESA (European Space Agency)

Prodex (BELSPO, Belgian Federal Government)

IAP VII/19 DYSCO (BELSPO, Belgian Federal Government)

ARC (Actions de Recherche Concertée)

Title: Haptic feedback of rebound improves predictive gaze tracking

Authors: ***F. SALMEN**[1,2], F. CREVECOEUR[1,2], J.-L. THONNARD[2,3], P. LEFEVRE[1,2]; [1]ICTEAM, Univ. catholique de Louvain, Louvain-la-Neuve, Belgium; [2]Inst. of Neurosci. (IoNS), Univ. catholique de Louvain, Brussels, Belgium; [3]Physical and Rehabil. Med. Dept., Cliniques Universitaires Saint-Luc, Brussels, Belgium

Abstract: Humans and animals rely on internal representation of the trajectories of moving stimuli, allowing predictive tracking despite transient target disappearance, or anticipation of future events. For instance, tennis players must predict the time and location of impact to achieve successful shots. An important question is whether the nervous system can integrate haptic feedback to improve the prediction about future events, such as the future trajectory of the ball following impact. To address this question, we studied predictive eye movements in a semi-static (fixed hand, moving target) collision task. Participants interacted with a robotic device

(KINARM, BKIN Technologies, Kingston) which applies force (i.e. haptic feedback of the collision) onto the participant's hand. Visual targets were projected onto a virtual reality display. Eye movement was recorded with an Eyelink camera (SR Research Ltd., Ottawa, Ontario, Canada). The participants (N=6) were instructed to perform visual tracking of round-shaped targets of different sizes before and after a collision with a bar representing the hand position. We assumed constant mass density, such that for a given velocity, a higher impact force was experienced for objects greater in size. The target was blanked for a constant period of 800ms immediately after the collision to address participants' capabilities to predict where the target would reappear. Two different object sizes and velocities resulted in four different impact forces and four different post-collision velocities due to simulated energy loss during the collision. The different conditions corresponded to a factorial design in which visual cues about target size and haptic feedback were provided or not. We then extracted the gaze position upon target reappearance to address participants' capabilities to integrate information about impact force in their predictive eye movements. Our results indicate that participants adjusted their gaze regarding to the target velocity after the collision. We found significant modulation of gaze position with target position at reappearance when both haptic feedback and visual cues about the target size were provided (linear regressions, $p<0.005$). In contrast, there was no significant correlation between gaze and target position at reappearance when no haptic feedback and no visual cue were available. Providing either haptic feedback or visual cue about target size evoked marginally significant correlation between gaze and target coordinate ($p\approx0.1$). We conclude that haptic feedback about impact force and visual cues are integrated in predictive tracking of a target after collision.

Disclosures: **F. Salmen:** None. **F. Crevecoeur:** None. **J. Thonnard:** None. **P. Lefevre:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.07/O43

Topic: D.03. Multisensory Systems

Support: NSERC Grant RGP355931

Title: Tool use and near-tool effects: Exploring the influence of training demands

Authors: ***G. E. TRACEY**, L. E. BROWN;
Psychology, Trent Univ., Peterborough, ON, Canada

Abstract: Learning to use a tool may involve the integration of multiple senses. Skilled tool users treat visual targets presented near a tool differently after training - these changes are called near-tool effects. The bimodal cell hypothesis explains this change in visual processing by

appealing to the activity of cells in the brain that respond both to visual and tactile stimuli. Visual receptive fields that respond to stimuli presented near the hand may expand down the length of the tool inducing near-tool effects. These effects may depend on active tool use. In this study we asked, can these effects be modulated by the demands placed on tool use? Eighty-one participants completed an experiment with a motor training task followed by a cross-modal interference task. Participants in tool-training groups were instructed to perform pointing movements with a tool held in their right hand. Five targets were presented along the mid-sagittal plane. Participants trained so that their movements were both quick and accurate to reach a criterion on 10/20 trials (the 50% training group) or 18/20 trials (the 90% training group). The control group received no active tool training. Participants would then complete a cross-modal interference task where tools were held in the left and right hands with index fingers placed on vibrotactile stimuli. Tactile stimuli were paired with seven visual stimuli presented near each tool. Stimulus pairs occurred on the same tool (compatible) or opposite tool (incompatible). Participants were instructed to press with their right foot on a keyboard as quickly as possible when they felt the tactile stimulus and reaction time (RT) was recorded. If near-tool effects are influenced by training demands, the size of the near-tool effect may vary with training demands. Our results indicate that our training manipulation was effective. The 90% group was more accurate and had lower movement times than the 50% group. However, a new group emerged who performed 180 trials of training but never reached criterion. They were less accurate and had slower movement times than the 50% training group and did not improve with practice. Results from the cross-modal interference task show that there were compatibility effects that appeared near the hand and near the tool tip that were not present for targets that were beyond the tool tip. There were no consistent differences between the 50% and 90% training groups. Near-tool effects depend on active tool-use in humans but do not appear to vary with training demands.

Disclosures: **G.E. Tracey:** None. **L.E. Brown:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.08/O44

Topic: D.03. Multisensory Systems

Support: DFG (SFB936/A3/B6)

EU (ERC-2010-AdG-269716)

Title: Large-scale cortical synchronization promotes multisensory processing

Authors: *F. GÖSCHL[1], P. WANG[1], U. FRIESE[1], P. KÖNIG[2], A. K. ENGEL[1];
[1]Dept. of Neurophysiol. and Pathophysiology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Inst. of Cognitive Sci., Univ. of Osnabrück, Osnabrück, Germany

Abstract: The integration of sensory signals from different modalities requires flexible interaction of remote brain areas. One candidate mechanism to establish local and long-range communication in the brain is transient synchronization of neural assemblies. In addition to the analysis of oscillatory power, assessment of the phase dynamics of multiple brain signals is a promising avenue to examine the integration of distributed information in multisensory networks. In the present study, human participants were engaged in a visuotactile pattern matching task while high-density EEG was recorded. To investigate the neural correlates of multisensory integration and assess effects of crossmodal stimulus congruence, we adapted an approach for purely data-driven analysis of neuronal coupling in source space that has recently been developed within our group. This method allows imaging of large-scale cortical networks in space, time and frequency without defining a priori constraints. We identified three clusters of interacting sources that synchronized in the beta-band (~ 20 Hz). The spatial and spectro-temporal profile of the first two clusters suggest an involvement in crossmodal sensory processing, whereas the third cluster appears to reflect decision-related processes. By directly relating coupling features to task performance, we demonstrate that the phase of neural coherence within the observed networks predicts behavior. Our results provide further evidence that neural synchronization is crucial for long-range communication in the brain and suggest a possible role of beta-band activity in multisensory integration.

Disclosures: F. Göschl: None. **P. Wang:** None. **U. Friese:** None. **P. König:** None. **A.K. Engel:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.09/O45

Topic: D.03. Multisensory Systems

Support: FNP Grant HomingPlus/2011-4/13

NCN Grant 2012/05/E/HS/03538

Marie Curie Career Integration Grant CIG – 618347

Title: Structural correlates of visual cortex plasticity in sighted braille learners

Authors: *L. BOLA[1,2], K. SIUDA-KRZYWICKA[1,4], M. PAPLINSKA[5], E. SUMERA[6], K. JEDNOROG[3], A. MARCHEWKA[2], M. ZIMMERMANN[7], M. SZWED[1];

[1]Jagiellonian Univ., Krakow, Poland; [2]Lab. of Brain Imaging, Neurobio. Ctr., [3]Lab. of Psychophysiology, Dept. of Neurophysiol., Nencki Inst. of Exptl. Biol., Warsaw, Poland; [4]École des Neurosciences Paris Île-de-France, Paris, France; [5]Acad. of Special Educ. in Warsaw, Warsaw, Poland; [6]Sch. for the Blind and Partially Sighted Children in Krakow, Krakow, Poland; [7]Fac. of Psychology, Univ. of Warsaw, Warsaw, Poland

Abstract: Neuroplasticity in the adult brain is thought to operate within the limits of sensory division, where the visual cortex processes visual stimuli and responds to visual training, the tactile cortex processes tactile stimuli and responds to tactile training, and so on. A departure from this rule is presumed possible only in sensory loss or brain injury - for example, the visual cortex of blind subjects can be recruited for braille reading. We recently showed that contrary to this presumption, the visual cortex of sighted braille learners becomes critical for tactile reading (Bola et al., SFN 2014). Here we wanted to test whether cross-modal plasticity during braille learning induces structural reorganization of the visual cortex. 29 subjects - mostly braille teachers and educators, naïve in tactile braille reading - were enrolled in a 9-month braille course. At the beginning of the course and at its end, they underwent a magnetic resonance imaging (MRI) session, during which high-quality T1-weighted images were acquired for voxel-based morphometry (VBM) analysis. Additionally, resting-state functional MRI (rsfMRI) data were collected for functional connectivity analysis. 9 months after the end of the course, 19 subjects participated in a follow-up MRI session. After the training we observed grey matter volume increase in the primary and secondary visual cortex, left premotor cortex and left cerebellum (lobules VII and VIII). Structural changes in the early visual cortex were left-lateralized and restricted to regions that represent peripheral visual field. The grey matter volume increases were preserved in a follow up scan, 9-months after the end of the training. Functional connectivity analysis showed that subparts of early visual cortex in which we observed structural plasticity were preferentially connected with primary somatosensory and motor cortices, even before the onset of the training. In line with our previous study (Bola et al., SFN 2014), we did not find structural changes in the somatosensory cortex. Our results show that tactile training can induce structural reorganization in the visual cortex. It is thus the first evidence that cross-modal, structural plasticity is possible in the healthy, adult brain. Together with our previous findings, our results suggest that this plasticity re-arranges the flow of tactile information in the brain. As its result, complex tactile input such as braille is transferred via corto-cortical connections from somatosensory to early visual cortices. That information is then subsequently processed in the high-level visual areas.

Disclosures: L. Bola: None. **K. Siuda-Krzywicka:** None. **M. Paplinska:** None. **E. Sumera:** None. **K. Jednorog:** None. **A. Marchewka:** None. **M. Zimmermann:** None. **M. Szwed:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.10/O46

Topic: D.03. Multisensory Systems

Title: Inducing sense of finger extension or retraction based on self-touch illusion and proprioception-vision correlation

Authors: K. MORI, Y. ISHIHIHARA, *K. KODAKA;
Grad. Sch. of Design & Archtecture, Nagoya City Univ., Nagoya-City / AICHI, Japan

Abstract: We can feel that both hands touch each other by providing a synchronized touch in self-touch illusion (STI, Ehrsson et al., 2005). It is well known that proprioceptive drift (PD) occurs in a manner that involves both hands attracting each other in STI. Although it is generally assumed that PD merely involves a horizontal movement of an entire hand, a thought experiment suggests that a finger extension also can fill a spatial gap between two hands (fingers). To the best of our understanding, though such an impression has been described by some participants in past experiments (Kodaka et al., 2014), no research to date has focused on inducing a finger extension in the body illusion. Our study developed a system inducing a sense of extension or retraction (SER) in a good combination of synchronized vibrations (for STI) and a vision-proprioception correlation. This physically regulates a distance between left and right index finger using two linear actuators (fixed to stands where hands are placed) and displays an animated finger image created by computer graphics (CG) through a head-mount display (HMD) that transforms with the movement of both hands. We performed two experiments with a total of 17 participants to measure the effect of synchronized vibrations and the visual correlation on sense of self-touch and SER in our system. There were eight kinds of conditions, each of which took 20 seconds and were repeated four times. The first factor concerned a specific modality's presentation; half of the presented conditions lacked a visual image in the HMD (Exp. 1) and synchronized vibrations (Exp. 2). In each trial with a visual image, the left index finger on CG was transformed in a manner involving extension for both fingers' separation and retraction for their approach; whereas, the length of the right index finger did not change on CG. The second factor concerned the average physical distance between both fingers. The participants were asked to answer some questions regarding STI and SER after each trial. Results indicated that lack of synchronized vibrations drastically and significantly worsened the subjective strength of STI and SRE (47% and 58% on average, respectively); whereas, the lack of a visual image worsened them slightly (80% each on average). This suggests that SRE is essentially based on an effort of the self-touch body image induced by synchronized vibrations. Another analysis found that the sense of retraction was significantly stronger when both hands were in close proximity; whereas, the sense of extension was significantly stronger when they were separated. Based on these results, the process of how SRE is induced with multiple sensor integration is discussed.

Disclosures: K. Mori: None. **Y. Ishihihara:** None. **K. Kodaka:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.11/O47

Topic: D.03. Multisensory Systems

Support: NIH/NICHD Grant 1R01HD071978-01A1

Title: Categorizing individuals by tactile, kinesthetic and visual impairments for individualized treatment for upper limb recovery post stroke

Authors: *D. GELLER[1], V. ALURU[1], S. BILALOGLU[1], Y. LU[2], P. RAGHAVAN[3]; [1]Rehabil., Rusk Rehabil. NYU Langone Med. Ctr., New York, NY; [2]New York Univ., New York, NY; [3]New York Univ. Sch. of Med., New York, NY

Abstract: Despite conventional rehabilitation, even relatively well recovered stroke survivors have persistent deficits in hand function that affects their quality of life. Hand function requires integration of sensory inputs with motor output. However, patients with stroke can have varying degree of impairment in tactile, kinesthetic and visual domains which can affect motor learning and recovery. We administered a battery of tests of impairment in tactile, kinesthetic and visual domains that pertain to grasping and lifting, and examined its effect on learning to adapt fingertip forces according to the texture, weight and shape of objects. Twenty subjects with stroke were categorized as having mild, moderate or severe impairments in the 3 sensory domains tested. Fingertip forces and movements during grasping and lifting were measured using force sensors and an instrumented glove. We found that while some subjects had deficits in all three domains, most subjects were impaired specifically in one domain. The impairment in adaptation of fingertip forces during grasping and lifting was directly correlated with the sensory impairment. For example, subjects with tactile deficits did not adapt their fingertip forces to object texture but could adapt their fingertip forces to object weight. Categorizing patients with stroke according to impairment type and severity may help plan individualized treatment programs for improved motor learning and recovery of upper limb function.

Disclosures: D. Geller: None. **V. Aluru:** None. **S. Bilaloglu:** None. **Y. Lu:** None. **P. Raghavan:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.12/O48

Topic: D.03. Multisensory Systems

Support: NINDS R01 NS078396

NSF BCS1358907

Title: Probing the neuroanatomy of olfactory processing in the human orbitofrontal cortex with electrical brain stimulation

Authors: *S. GATTAS, J. YIH, J. PARVIZI;
Lab. of Behavioral and Cognitive Neurosci., Stanford Univ., Stanford, CA

Abstract: Several functional magnetic resonance imaging (fMRI) studies in humans and electrophysiological studies in non-human primates have shown olfaction-related activity in the orbitofrontal cortex (OFC). To probe the causal role of various anatomical OFC subregions in olfaction, we used electrical brain stimulation (EBS) in patients implanted with unilateral subdural electrodes in the right or left OFC. We hypothesized that EBS in specific subregions of the OFC would elicit olfactory hallucinations, and predicted that the valence of subjective olfaction hallucinations would be altered by varying electrical charge density. We included data from 10 patients (6 right hemisphere) with medically intractable epilepsy. In all subjects, the OFC was identified to be outside the ictal onset zone. Right hemisphere group coverage consisted of 75 OFC electrodes (68 stimulated), whereas the left contained 84 (73 stimulated). The EBS procedure involved the delivery of current to adjacent electrode pairs at varying combinations of amplitudes and frequencies, interspersed with sham trials in which no current was delivered. The patients' post-stimulation subjective experiences were categorized as smell, taste, smell and taste, or other effects. Since smell and taste effects were often reported in conjunction, we ultimately grouped these effects into one category. The valence of each evoked effect was then categorized as positive, negative, or neutral. From a bilateral sample of stimulated electrodes, 17.02% evoked an olfaction-gustation effect. Moreover, 93.33% (14/15) of right and 77.78% (7/9) of left hemisphere electrodes with olfaction-gustation effects were located in central-to-posterior OFC. More specifically, electrodes were positioned within the following anatomical subregions; bilateral gyrus rectus (5/8 subjects), bilateral medial orbital gyri (3/9 subjects), the caudal region of the right posterior orbital gyrus (1/3 subjects), and the right lateral orbital gyrus (1/5 subjects). There was no anatomically consistent pattern observed in elicited valence effects. However, increases in frequency or amplitude of electrical stimulation resulted in a greater intensity of the hallucination experienced. The present study provides causal evidence that OFC subregions involved in olfaction-gustation are within central-to-posterior OFC. Lastly, it provides insight on the effect of stimulation parameters on the intensity of the experienced hallucination.

Disclosures: **S. Gattas:** None. **J. Yih:** None. **J. Parvizi:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.13/P1

Topic: D.03. Multisensory Systems

Title: Coupled bimanual arm training for individuals with severe hemiparesis

Authors: *P. THAI[1], V. ALURU[2], S. MILANI[3], A. JOHNSON[4], A. TANG[2], D. GELLER[2], S. BILALOGLU[2], D. WEISZ[5], Y. LU[6], P. RAGHAVAN[2];
[1]New York Univ. Langone Med. Ctr., New York, NY; [2]Rehabil. Med., New York Univ. Sch. of Med., New York, NY; [3]Dept. of Med., Richmond Univ. Med. Ctr., Staten Island, NY; [4]Intrnl. Med., Yale Sch. of Med., Waterbury, CT; [5]Mount Sinai, New York, NY; [6]Steinhardt Sch. of Culture, Educ. and Human Develop., New York Univ., New York, NY

Abstract: Few options exist for training arm movements in subjects with hemiparesis when they have little active range of motion. An inability to move often leads to non-use, stalled recovery and progressive upper limb deformity. The purpose of this study was to test the safety and feasibility of training individuals with hemiparesis with a non-powered device, the Bimanual Arm Trainer, that facilitates coupled bimanual training of shoulder external rotation and elbow extension. Nine subjects with post-stroke hemiparesis, preserved passive range of motion in shoulder, elbow and wrist joints and minimal spasticity, trained with the device twice a week for six weeks. All subjects tolerated the training and no adverse events were reported. Motor impairment on the upper extremity Fugl-Meyer Scale and active range of motion measured using motion analysis techniques were assessed pre- and post-training. Subjects showed significant improvement in the Fugl-Meyer score, particularly for proximal movements of the shoulder and elbow with changes in the flexor synergy pattern. Changes in active range of motion in the paretic limb for both trained and untrained movements corroborated with the improvement in Fugl-Meyer scores. The results demonstrate the safety and feasibility of using the Bimanual Arm Trainer to facilitate motor recovery in individuals with severe hemiparesis.

Disclosures: P. Thai: None. **V. Aluru:** F. Consulting Fees (e.g., advisory boards); Mirrored Motion Works. **S. Milani:** None. **A. Johnson:** None. **A. Tang:** None. **D. Geller:** None. **S. Bilaloglu:** None. **D. Weisz:** None. **Y. Lu:** None. **P. Raghavan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mirrored Motion Works.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.14/P2

Topic: D.03. Multisensory Systems

Support: JSPS KAKENHI Grant 26120007

Title: Mental transformation of body parts in manipulating the somatotopic representation

Authors: ***T. SUDO**[1], Y. OOUCHIDA[1], S.-I. IZUMI[1], K. MOGI[2];
[1]Tohoku Univ., Sendai, Miyagi, Japan; [2]Sony Computer Sci. Laboratories, Inc., Tokyo, Japan

Abstract: The embodied representations of self and others play an important part in social interaction such as perspective taking, imitation, and theory-of-mind mechanisms. In order to perceive one's own body and their environment, and achieve the intended movements successfully, the brain would require integrating multisensory information consisting of proprioceptive information about the location and the current body position, as well as the external signals from visual information. Perspective taking through the manipulation of own body image is dependent on the comparison between the representations of self and others, involving a transformation of egocentric perspective to the allocentric one. Rotation symmetry, in which the subject navigates egocentric image of the self for the destination (Keehner et al, 2006), is one way of transformation between the representation of self and others. Recent studies have suggested that the rotation symmetry in the mental transformation of one's own body correlates with a similar tendency of object mental rotation (Sekiyama 1982; Parsons 1987; Zacks 2008). During the mental rotation of body parts, subjects might change their perspectives as if in a task of object mental rotation. Such a transformation process would also require integrating multisensory information consisting of visual and proprioceptive information. On the other hand, several studies revealed that, when limbs are crossed, the integration of tactile with proprioceptive information is hindered and tactile localization becomes less accurate (Heed et al, 2014). Here we have conducted some consecutive experiments with hand mental rotation paradigm in which we manipulated the hand posture and location of visual stimuli in order to examine the effect of misalignment of somatotopic and external reference frames. The subjects were instructed to make a few perceptual judgments on spatial configurations in the mental rotation paradigm with various alignments of their own hand and presentation stimuli. Behavioral data from this experiment suggest the robustness of integration of sensory information in the mental imagery of bodily self. Based on the results, we discuss the significance of crossmodal congruency in the fundamental aspects of bodily self-consciousness.

Disclosures: **T. Sudo:** None. **Y. Oouchida:** None. **S. Izumi:** None. **K. Mogi:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.15/P3

Topic: D.03. Multisensory Systems

Support: The Swedish Research Council

The James McDonnell Foundation

Söderbergska Stiftelsen

Riksbankens Jubileumsfond

Title: The magnetic touch illusion: a proposed perceptual correlate of peripersonal space

Authors: *A. GUTERSTAM, H. ZEBERG, V. MENDERES OZCIFTCI, H. EHRSSON; Karolinska Institutet, Stockholm, Sweden

Abstract: To accurately localize our limbs and guide movements toward external objects, the brain needs to represent the body and its surrounding (peripersonal) visual space. Although we do not perceive the space near our body as "special", single-unit recordings in monkeys have identified visuo-tactile-proprioceptive neurons in specific multisensory regions that represent peripersonal space in body-part-centered reference frames. In humans, the underlying multisensory integrative process has been implicated in limb self-attribution based on studies on the classical rubber hand illusion. In this study, we show that the application of brushstrokes in mid-air at some distance above a rubber hand - but never actually touching it - in synchrony with brushstrokes on the hidden real hand results in the illusory sensation of a "magnetic force" between the brush and the hand, which is strongly correlated with experienced rubber hand ownership. In eight experiments including a total of 101 healthy volunteers (60 females, mean age 27±5 years), we characterized this "magnetic touch illusion" using motion tracking (Polhemus FASTRAK, Vermont, USA) of the brush moving in mid-air and measuring questionnaire responses, proprioceptive drift, or real-time illusion vividness ratings. We found that the magnetic touch illusion exhibits striking similarities to the visual receptive field properties of peripersonal space neurons, featuring a non-linear decay in illusion strength at 40 cm ($p=0.01$) that is not significantly affected by gaze direction ($p>0.05$) (Experiment 1a, 1b, and 1c) and follows changes in rubber hand position (Experiment 4). Furthermore, we show that the mere expectation of a tactile event on the rubber hand does not contribute to the illusion experience (Experiment 2a and 2b) and that the "magnetic force" does not penetrate physical barriers (Experiment 3a and 3b). These findings provide strong support for the notion that multisensory integration within peripersonal space is an underlying mechanism for bodily self-attribution. Moreover, we propose that the magnetic touch illusion constitutes the first example of a perceptual correlate of peripersonal space in humans. As such, our findings provide a novel prediction for neurophysiological studies of peripersonal space, namely that that the visual receptive field of perihand neurons will remap with the introduction of a physical barrier close to the hand.

Disclosures: A. Guterstam: None. **H. Zeberg:** None. **V. Menderes Ozciftci:** None. **H. Ehrsson:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.16/P4

Topic: D.03. Multisensory Systems

Support: MEXT/JSPS grants (#23300151, #15H03126)

a MHLW grant (BMI)

Title: A rubber hand experiment using an EMG controlled robotic arm in amputee participants

Authors: *Y. SATO[1], T. KAWASE[1], K. TAKANO[1], K. KANSAKU[1,2];
[1]Sys. Neurosci. Sect., Dept. of Rehab. for Brain Funct., Res. Inst. of Natl. Rehabil. Ctr., Tokorozawa, Japan; [2]Brain Sci. Inspired Life Support Res. Ctr., The Univ. of Electro-Communications, Chofu, Japan

Abstract: Feeling ownership of our limbs represents a fundamental aspect of self-consciousness, and in some circumstances, the feeling is extended out of our own body, as in the rubber hand illusion (Botvinick and Cohen, 1998). In our previous rubber hand experiment in able-bodied participants, we used an in-house electromyography (EMG) controlled robotic arm, and evaluated sense of agency (SA) and sense of ownership (SO) by subjective ratings (-3 to +3) (Kalckert & Ehrsson, 2014). We found that subjective ratings (SA/SO) in able-bodied participants were significantly greater than 0 when the robotic arm was synchronously moved with their arm (Sato et al., 2015). In this study we performed the rubber hand experiment using the EMG controlled robotic arm in amputee participants (n=3). The robotic arm was placed in front of the amputee participants. A cloth was placed to cover the stump of the participant's amputated arm. EMG signals were recorded from the participants' amputated arm to control the robotic arm. The wrist of the robotic arm was flexed/extended when the participant's wrist flexors/extensors were contracted. After the experiment, SA and SO were evaluated by subjective ratings (-3 to +3). The experiment was repeated six times. Average value of the subjective ratings (SA/SO) in the 3 amputee participants were 2.2±0.7/2.1±0.6, 1.4±0.1/0.9±0.1, and 1.8±0.4/2.4±0.4, respectively. The subjective ratings (SA/SO) in the participants were significantly greater than 0 ($p < 0.05$). These results suggest that rubber hand illusion-like experience was induced by using an EMG controlled robotic arm in the amputee participants.

Disclosures: Y. Sato: None. **T. Kawase:** None. **K. Takano:** None. **K. Kansaku:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.17/P5

Topic: D.03. Multisensory Systems

Support: KAKENHI 25350776

KAKENHI 26120002

Title: Interactions between agency and ownership by moving virtual hand illusion

Authors: *S. UNENAKA, S. SHIBUYA, Y. OHKI;
Depatment of Integrative Physiol., Kyorin Univ. Sch. of Med., Tokyo, Japan

Abstract: Previous studies on bodily self-consciousness have reported some interactions between senses of agency and ownership of a moving rubber hand. However, these interactions were mainly observed in a subjective measurement (i.e. retrospective self-report), but not in an objective one (i.e. proprioceptive drift). We examined the interactions in both the self-report and the proprioceptive drifts using a moving virtual hand illusion. Normal subjects (n=20) placed their right hand on a 2D manipulandum with a low-friction. The hand was not visible directly, but an image of the life-size virtual right hand was displayed 12 cm in front of the real right hand. To induce the virtual hand illusion, subjects were required to move the virtual hand cyclically by manipulating their real hand. A 2 x 2 factorial design was applied to dissociate the senses of agency and ownership: movement types (active and passive) and virtual hand positions (anatomically congruent and incongruent). Subjects' hands were moved passively in the passive conditions, and the virtual hand was rotated at 180 degrees in the incongruent conditions. As the objective measurement of the ownership, proprioceptive drifts were estimated from three tests before and after illusion induction: bimanual arm position matching (BM), visual positional judgment (VJ), and target-reaching movement (TR). Senses of agency and ownership during illusion induction were subjectively measured using a Likert rating-scale questionnaire. Ratings on the questionnaire showed a double dissociation of agency and ownership. Proprioceptive drifts changed differently between the tests. They were observed only in the congruent conditions in BM, similar to the ownership rating. On the other hand, those in VJ and TR were influenced by both movement type and hand position, and were the greatest in the congruent active condition. Proprioceptive drifts obtained from three tests were weakly correlated ($r>0.24$, $p<0.05$), except for those between TR and BM ($r=0.06$). We further performed multiple regression analyses between proprioceptive drifts from three tests (independent variables) and the questionnaire ratings (dependent variables). Results revealed that only the ownership rating ($r=0.42$, $p<0.05$) could be explained by drifts in BM ($\beta=0.29$, $p<0.05$). These results suggest that agency could affect objective ownership measurements, but in different manners between tests; agency can increase proprioceptive drifts only in VJ and TR. On the other hand, in the current study, the subjective ownership measurement was not influenced by agency, which may be reflected in proprioceptive drifts in BM.

Disclosures: S. Unenaka: None. **S. Shibuya:** None. **Y. Ohki:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.18/P6

Topic: D.03. Multisensory Systems

Support: NIH - R01EB009233

NIH - R21HD057512

NIH - R01NS078223

K01 - MH103594

McDonnell Center for Systems Neuroscience

Title: Functional mapping of High-Density Diffuse Optical Tomography using movie viewing

Authors: *A. K. FISHELL[1,2], A. T. EGGEBRECHT[1], S. E. PETERSEN[1,3], J. P. CULVER[1,4]; [1]Radiology, [2]Div. of Biol. and Biomed. Sci., [3]Neurol., Washington Univ. Sch. of Med., Saint Louis, MO; [4]Physics, Washington Univ., Saint Louis, MO

Abstract: fMRI has previously shown that cortical responses to movies are highly reproducible, both within and across viewers. In the present study, we asked two questions related to this observation. First, does an optical neuroimaging technique, High-Density Diffuse Optical Tomography (HD-DOT), have sufficient resolution and field-of-view to replicate this result? Second, can movie-evoked responses be used to functionally map cortical areas? To answer these questions, healthy human participants (N=9; 6 Female; Age range: 22-33) viewed a 10 min clip from The Good, The Bad, and The Ugly) while being scanned with a HD-DOT neuroimaging system. We observed high response reproducibility across broad portions of the cortex, both within and across participants. Using an MR-derived pacellation scheme to identify cortical regions, we found that response reproducibility is particularly elevated in visual and auditory regions, recapitulating results obtained using fMRI. Further, we demonstrate the feasibility of using movie stimuli as a tool for mapping functional areas. Specifically, we observed considerable overlap between movie-evoked responses and responses evoked by more traditional localizer paradigms (e.g. passive word listening, retinotopy), indicating that passive movie viewing is a simple paradigm that maps many of the same areas as multiple functional localizers. Finally, we found that the topography of response reproducibility can be modulated by movie content. Participants also viewed a second movie with extremely strong motion content. In comparison to the first stimulus, this “motion intensive” film generated reproducibility maps with greater response reproducibility in motion sensitive area MT, suggesting that movie content can be tailored to localize specific regions of interest. From these results, we conclude that: (1) HD-DOT has sufficient resolution, field-of-view, and signal-to-noise to detect reproducible movie-evoked responses, and (2) movie-evoked responses can serve as a tool for mapping functional areas related to visual and auditory processing.

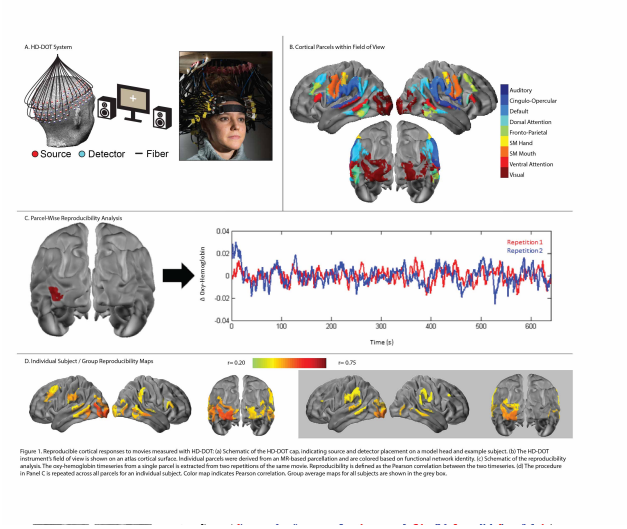


Figure 1. Reproducible cortical responses to movies measured with HD-DOT. (a) Schematic of the HD-DOT cap, indicating source and detector placement on a model head and example subject. (b) The HD-DOT instrument's field of view is shown on an atlas cortical surface. Individual parcels were derived from an MR-based parcellation and are colored based on functional network identity. (c) Schematic of the reproducibility analysis. The oxy-hemoglobin timeseries from a single parcel is extracted from two repetitions of the same movie. Reproducibility is defined as the Pearson correlation between the two timeseries. (d) The procedure in Panel C is repeated across all parcels for an individual subject. Color map indicates Pearson correlation. Group average maps for all subjects are shown in the grey box.

Disclosures: **A.K. Fishell:** None. **A.T. Eggebrecht:** None. **S.E. Petersen:** None. **J.P. Culver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cephalogics LLC.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.19/P7

Topic: D.03. Multisensory Systems

Title: Multisensory integration varies with target and environment complexity in a virtual environment: towards a naturalistic model of multisensory integration

Authors: H. D. BAILEY[1], A. B. MULLANEY[2], K. D. GIBNEY[3], *L. D. KWAKYE[1]; [1]Neurosci., Oberlin Col., Oberlin, OH; [2]Great Sci. Acad., Cleveland, OH; [3]Vanderbilt Univ., Nashville, TN

Abstract: We are continually bombarded by information arriving to each of our senses; however, the brain seems to effortlessly integrate the separate information into a unified percept. Although multisensory integration has been researched extensively using simple computer tasks and stimuli, much less is known about how multisensory integration functions in a real world context. Virtual reality offers the perfect combination of realism and precise control over the environment to investigate this question. We have chosen to begin this area of investigation with a task known as the detection task (redundant target effects). In its computer game version, participants are asked to detect a white circle, white noise burst, or a combination of the two as fast as possible. Participants are faster at detecting multisensory targets than either of the

unisensory targets. In the virtual reality version of this task, participants detected targets varying in complexity within a virtual world that also varied in complexity. The environmental complexity was modulated using the three following virtual worlds: a gray room that contains no depth cues, texture, color, or environmental sounds; a room with textured walls that offers depth cues, soft background white noise, but no color or environmental context; a room that mimics the experimental testing room that offers depth cues, texture, color, and identifiable background noise. The target complexity was modulated using the following three conditions: white circle with no 3D shading and/or white noise burst; white circle with 3D shading thus appearing as a white ball and/or noise which is modulated in amplitude and frequency; tennis ball with 3D shading and color and/or a tennis ball sound. Using the geometric measure of Miller's inequality proposed by Colonius and Diederich (2006), we demonstrated integrative effects in every environment-target pairing and further showed that the degree of integration positively correlates with target complexity but only in our most complex environment. To further investigate this effect of target complexity on integration, we conducted a second experiment to examine the effect of target unpredictability. The effect of target complexity observed in the first experiment did not hold when the target identity or location were unpredictable; however, we were able to observe integrative effects in every condition. Our study is the first to definitively show that minimal and more naturalistic stimuli elicit comparable redundant target effects but that the features of the environment and target modulate the degree of integration.

Disclosures: H.D. Bailey: None. **A.B. Mullaney:** None. **K.D. Gibney:** None. **L.D. Kwakye:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.20/P8

Topic: D.03. Multisensory Systems

Support: NIH/ NICHD grant 1R01HD071978-01A1

Title: Adaptation of grip forces to tactile surfaces is based on categorization of frictional surfaces rather than on coefficient of friction

Authors: *S. BILALOGLU[1], Y. LU[2], V. ALURU[3], D. GELLER[3], P. RAGHAVAN[3]; [1]NYU Langone Med. Ctr., New York, NY; [2]Steinhardt Sch. of Culture, Educ. and Human Develop., New York Univ., New York, NY; [3]Dept. of Rehabil. Med., New York Univ. Sch. of Med., New York, NY

Abstract: Adaptation of fingertip forces to the friction at the grip surface is necessary to prevent use of inadequate or excessive grip forces. The purpose of this study was to understand the

interaction between the frictional surfaces and the fingertip grip surface during grasping and lifting. The coefficients of friction (COF) of 18 different frictional surfaces were obtained by dragging the textured surface affixed to a standard mass across a standard surface on a tabletop. The COF ranged from 0.36 to 1.17 (mean=0.73). Ten subjects without neurologic deficits then grasped and lifted an instrumented grip device with the 18 different surfaces 7 times for each surface with bare hands and with a thin layer of tegaderm applied to the fingertip (to control for subject-level variability in fingertip moisture and skin texture). There were no significant differences in 2-point discrimination or pressure sensitivity threshold between barehands and tegaderm, but tegaderm impaired static tactile discrimination. The COF during interaction of the frictional surface with the fingertips was measured as the inverse of the slip ratio (grip force/ load force at the moment of slip). As expected, there was greater between-subject variability in the slip ration with bare hands (ICC=0.67) than with tegaderm (ICC=0.63). However, instead of a monotonic change in the slip ratios with the COF of the textured surfaces (measured against a standard surface), the slip ratios clustered into smooth and rough categories both with bare hands and tegaderm. We therefore collapsed all the smooth and rough surfaces (9 each) and further examined the adaptation of fingertip grip force rates (PGFR) to the frictional surface. We found that the average PGFR was significantly higher for the smooth surfaces compared with the rough surfaces ($b=-5.75$, $p<0.001$) with bare hands but not with tegaderm ($b=-1.26$, $p=0.1932$). The PGFR did not vary with the COF. These results suggest that the tactile surfaces are categorized broadly as smooth and rough, and the adaptation of fingertip forces is based on this broad categorization rather than on the coefficient of friction of the tactile-surfaces per se. Using tegaderm to remove subject-level variability in fingertip moisture and skin texture effectively impairs tactile discrimination and adaptation of grip forces. The receptors on the skin surface necessary for tactile discrimination are also necessary for grip force adaption.

Disclosures: S. Bilaloglu: None. **Y. Lu:** None. **V. Aluru:** None. **D. Geller:** None. **P. Raghavan:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.21/P9

Topic: D.03. Multisensory Systems

Support: MEXT KAKENHI 25135720

JSPS KAKENHI 15H01764

Title: Shared neural representation for visual and tactile material property

Authors: *H. YAMASHIRO[1], H. YAMAMOTO[2], C. ISAMI[3], S. SUKIGARA[3], T. MURASE[4], M. UMEDA[4], T. HIGUCHI[4];
[1]Aino Univ., Ibaraki-Shi, Osaka, Japan; [2]Grad. Sch. of Human and Envrn. Studies, Kyoto Univ., Kyoto-shi, Kyoto, Japan; [3]Kyoto Inst. of Technol., Kyoto-shi, Kyoto, Japan; [4]Meiji Univ. of Integrative Med., Nantan-shi, Kyoto, Japan

Abstract: Humans can perceive tactile property of objects by just seeing them, such that we can see the fluffiness of fur or the smoothness of leather. However, the neural basis of such visuo-tactile information transformation are not fully understood. We hypothesized that such processes involve cooperative action of visual and tactile systems and common neural representations of material properties of objects. To explore such representations, we measured brain activity using functional magnetic resonance imaging (fMRI), while subjects viewed or touched pieces of cloth. Fifteen naïve subjects participated in the experiment. The stimuli were a soft wool cloth or a hard denim cloth, which were presented visually or tactually. In each visual trial, subjects viewed a video presented to their right eye for four seconds, which recorded a rotating piece of cloth stuck onto a sinusoidal wavy surface from an oblique viewpoint, so that the texture of the cloth can be seen clearly. In each tactile trial, subjects repeatedly grasped one of these pieces of cloth for four seconds, which was put onto their right hand and removed by the experimenter. During a three minute run, each stimulus was presented for three times in random order with random inter-stimulus intervals. Each subject was scanned for ten runs. During the scan, subjects performed a one-back task comparing the hardness/softness of the stimulus, in which they reported whether the current stimulus is harder/softer, or same as previous one, by pressing buttons. Surface based searchlight analysis revealed the cross-modal nature of the natural texture perception. On one hand, visual texture representations were found in somatosensory and association cortices as well as visual cortex. On the other hand, tactile texture representations were found in visual cortex and association cortices as well as somatosensory cortex. Furthermore, shared visuo-tactile representations were found in parietal association, somatosensory, and visual cortices. These results suggest that texture information are transferred across functionally segregated sensory and associative brain regions.

Disclosures: H. Yamashiro: None. **H. Yamamoto:** None. **C. Isami:** None. **S. Sukigara:** None. **T. Murase:** None. **M. Umeda:** None. **T. Higuchi:** None.

Poster

329. Cross-Modal Processing in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 329.22/P10

Topic: D.03. Multisensory Systems

Support: CIHR fellowship

Title: Interactions between posterior parietal and primary motor cortices are differentially modulated after the rubber hand illusion with ageing

Authors: *R. ISAYAMA[1,2], M. VESIA[1], G. JEGATHEESWARAN[1,2], B. ELAHI[2], C. GUNRAJ[1], L. CARDINALI[3,4], A. FÁRNE[4], R. CHEN[1];
[1]Div. of Brain, Imaging and Behavior - Systems Neurosci., Toronto Western Res. Inst., Toronto, ON, Canada; [2]Univ. of Toronto, Toronto, ON, Canada; [3]The Brain and Mind Inst., Univ. of Western Ontario, London, ON, Canada; [4]Lyon Neurosci. Res. Ctr., Lyon, France

Abstract: Background:Ageing affects multiple systems in the brain, including the motor and sensory systems. Several studies have shown that older adults, when compared to younger adults, showed greater shortening of response time to multi-modal than to single-modal sensory cues, suggesting that ageing influences multi-modal sensory integration. However, how ageing affects the neural underpinnings for multi-modal sensory integration, particularly the interaction between posterior parietal cortex (PPC) and primary motor cortex (M1), remains unclear. The objective of this study is to evaluate in older and young adults 1) multi-modal sensory integration at the behavioral level and 2) the changes in the interactions between PPC and M1 with multi-modal sensory integration. We hypothesize that multi-modal sensory integration will be enhanced and will more strongly modify the PPC-M1 interaction in older than young adults. Methods:We tested 10 healthy young (age:24.7±2.6 years) and 9 older adults (age:66.3±5.7 years). Multi-modal sensory integration was examined by the rubber hand illusion (RHI) paradigm. Subjects viewed a rubber hand being stroked by a brush during the application of synchronous (test condition) or asynchronous (control condition) brush strokes on their own unseen hand. The level of RHI was determined with a questionnaire assessing the subject's feeling of ownership of the rubber hand and the drift in the estimation of his/her own hand position. Transcranial magnetic stimulation (TMS) in a paired-pulse paradigm was used to test the interactions between left PPC and left M1. Conditioning stimulus was delivered to the PPC 4, 6, 8, and 10 ms prior to the test stimulus to M1. Motor evoked potentials (MEPs) were recorded from hand muscles at rest before and immediately after RHI induction. PPC-M1 interaction was assessed by comparing the conditioned to the unconditioned (TMS to M1 alone) MEP amplitudes. Results:Both groups exhibited stronger rubber hand ownership in the synchronous than the asynchronous condition, but the difference was greater in younger adults due to stronger ownership in older adults in the asynchronous condition. PPC-M1 interactions were similar between young and older adults at baseline. However, in older but not in younger adults, PPC-M1 interaction was facilitated in the synchronous and inhibited in the asynchronous condition at 6 ms ISI. Conclusions:RHI was easier to be elicited and was associated with greater modulation of the PPC-M1 interaction in older than younger adults. These findings may reflect enhanced multi-modal sensory integration in older adults, leading to faster responses to multi-modal sensory stimuli.

Disclosures: R. Isayama: None. **M. Vesia:** None. **G. Jegatheeswaran:** None. **B. Elahi:** None. **C. Gunraj:** None. **L. Cardinali:** None. **A. Fárne:** None. **R. Chen:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.01/P11

Topic: D.04. Vision

Title: Mechanisms underlying orientation selectivity in the mouse retina

Authors: *A. NATH, G. W. SCHWARTZ;
Northwestern Univ., Chicago, IL

Abstract: Orientation selectivity (OS) is well known in the visual cortex (Hubel and Wiesel 1962) for decades and has recently been found in mouse lateral geniculate nucleus (Marshel et. al. 2012; Zhao et. al. 2013; Piscopo et. al. 2013) and superior colliculus (Feinberg and Meister 2015, Ahmadlou and Heimel 2015). Does this property primarily arise in these regions or is there an orientation selective input from the retina via retinal ganglion cells (RGCs)? Orientation selective RGCs have been reported in the rabbit retina (Levick, 1967; Caldwell et. al. 1978; Amthor, 1989; Bloomfield 1994; Venkataramani and Taylor, 2010) but the underlying neural mechanisms remain unclear. The goal of our research was to find and characterize OS cells in the mouse retina and probe the circuit mechanisms that underlie orientation selectivity. We probed RGCs with flashed and moving bars, and drifting gratings in an *ex vivo* preparation of the intact retina. We characterized light responses in both cell-attached and whole-cell electrophysiological recordings. To correlate physiology with morphology, we imaged cells with two photon and confocal microscopy. We found OS RGCs aligned with the two cardinal axes of the mouse visual system, and we observed OS in both excitatory and inhibitory synaptic inputs to RGCs, in contrast with previous reports (Levick, 1967; Caldwell et. al. 1978; Bloomfield, 1994). Horizontal OS RGCs have horizontally oriented dendritic fields whereas vertical OS RGCs are symmetric in dendritic morphology. Pharmacology revealed that the inhibitory OS pathway is glycinergic. Moreover, this inhibition is oppositely oriented to the excitation in horizontal OS RGCs. However, vertical OS RGCs receive OS inhibition in random orientations. Amacrine cells performing neuritic OS computation might be the source of such inhibition. Additionally, horizontal OS cells might selectively wire with these amacrine cells whereas vertical OS RGCs wire randomly. Computational modeling coupled with pharmacology suggests that OS excitation might arise due to presynaptic inhibition in both kinds of cells. With the power of genetic tools available in mouse, our identification of OS RGCs in this species will lead to future work on the processing of OS circuitry and information throughout the visual system.

Disclosures: **A. Nath:** None. **G.W. Schwartz:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.02/P12

Topic: D.04. Vision

Title: Control of excitation-inhibition balance in the directionally selective circuit in mouse retina by nicotinic acetylcholine receptors

Authors: *A. HOGGARTH, S. SETHURAMANUJAM, V. JAIN, A. MCLAUGHLIN, G. B. AWATRAMANI;
Dept. of Biol., Univ. of Victoria, Victoria, BC, Canada

Abstract: Introduction: The precise excitation-inhibition (E/I) balance is a key determining factor of direction selectivity (DS) in the mammalian retina. Acetylcholine (ACh) and GABA released from starburst amacrine cells (SACs) are both thought to act postsynaptically on DS ganglion cells (DSGCs), through the activation of nicotinic ACh receptors (nAChRs) and GABAA ionotropic receptors. Here, we examine the role of presynaptic nAChRs in facilitating GABA release from SACs. Methods: We combined electrophysiology, pharmacology and optogenetic techniques to investigate the role of nAChRs in modulating release of GABA from SACs. DSGCs and SACs were identified in labelled transgenic mouse lines (Hb9::eGFP and ChAT-Cre:Ai9, respectively) for whole-cell and extracellular patch-clamp recordings using 2-photon laser scanning microscopy. Specific expression of channelrhodposin (ChR2) in SACs was attained using the ChAT-Cre promoter and allowed us to selectively examine the output function of these cells. Responses to moving spots of light and moving sine wave grating stimuli were recorded. Results: Consistent with previous studies we found that application of nAChR antagonists (100 μM hexamethonium; Hex) reduced the DSGC's 'preferred' spiking response. Surprisingly, however, we also noted that Hex did not strongly affect the weaker 'null' response. To understand the lack of effect on null direction spiking responses we next performed voltage-clamp experiments. Interestingly, the strength of null direction inhibition mediated by SACs (monitored as inhibitory currents DSGCs voltage clamped at 0 mV) was reversibly reduced by Hex (~ 40 % reduction in peak amplitude). This was likely an effect on SACs, as a similar decrease in the IPSC was observed when SACs were directly stimulated optogenetically (in the presence of agents that occluded photoreceptor responses). In contrast to SAC output, the inputs to SACs were not affected by Hex. Thus, we posit that activation of nAChRs at distal SAC dendrites (output sites) leads to a facilitation of GABAergic release. The reduced null inhibition is consistent with the relative increase in the observed null-direction spiking response in the presence of Hex, a reflection a perturbed E/I balance. Conclusions: ACh release from SACs activates both post- (on DSGCs) as well as presynaptic nAChRs (on SACs) thereby controlling E/I balance. Together, the endogenous activation of pre- and postsynaptic nAChRs serves to sharpen directional tuning in DSGCs.

Disclosures: A. Hoggarth: None. **S. Sethuramanujam:** None. **V. Jain:** None. **A. McLaughlin:** None. **G.B. Awatramani:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.03/P13

Topic: D.04. Vision

Title: Spatiotemporal receptive field properties of starburst amacrine cell dendrites

Authors: *H. DING, J. DIAMOND;
NIH, Bethesda, MD

Abstract: Dendritic computation is a fundamental aspect of neuronal information processing, particularly in retinal amacrine cells, in which synaptic inputs and outputs occur in the same dendrites. Separate dendritic branches of starburst amacrine cells (SACs) process visual information independently to generate direction selectivity (DS) in the mammalian retina. Despite a detailed understanding of the connectivity within the DS circuitry, however, the mechanisms underlying DS computation in SACs remains unclear. Recent connectomic findings suggest that spatiotemporal properties of excitatory inputs give rise to direction selectivity in SACs. To examine the functional consequences of this proposed model, we measured the spatiotemporal input-output relationship along individual SAC dendrite by mapping the visual receptive field of presynaptic terminals. Using two-photon fluorescence imaging, we recorded intracellular calcium activity in OFF starburst amacrine cells and mapped the receptive fields of varicosities in the distal region of dendrites where the GABAergic output synapses are located. Anatomical reports indicate that excitatory inputs are located along dendrites proximal to (and possibly overlapping with) inhibitory outputs, suggesting that a spatial offset should exist between the location of a synaptic varicosity and its visual receptive field, a prediction that is confirmed by our experiments. In addition, published connectomic data predict that the temporal characteristics of the receptive field should vary with distance to the soma, with proximal inputs being slower. Our preliminary results indicate temporally heterogeneous responses in SAC varicosities, and further analysis is required to determine whether these differenced correspond to specific regions of the receptive field. We also have measured the response variability within single output synapses and the correlation between multiple synapses along the same dendritic branch to identify factors that limit DS tuning in SACs.

Disclosures: H. Ding: None. **J. Diamond:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.04/P14

Topic: D.04. Vision

Support: NSF IGERT graduate fellowship

NEI Grant EY022933

Pew Charitable Trusts

McKnight Endowment Fund for Neuroscience

Karl Kirchgessner Foundation

Alfred P. Sloan Foundation

Title: Distinct inhibitory spatial scales improve information transmission in the retina

Authors: *L. T. MCINTOSH[1], M. MANU[3], D. B. KASTNER[4], B. NAECKER[1], S. A. BACCUS[2];
[1]Neurosciences Grad. Program, [2]Neurobio., Stanford Univ., Stanford, CA; [3]Dept. of Neurosurg., Med. Sch. Hannover, Hannover, Germany; [4]Psychiatry, UCSF Sch. of Med., San Francisco, CA

Abstract: All visual information in the brain derives from the responses of retinal ganglion cells, which receive photoreceptor signals that have been pooled and filtered by parallel excitatory and inhibitory pathways. One prominent theory of retinal function, efficient coding theory, states that ganglion cell responses maximize information about the visual environment subject to an energy constraint. As support for this theory, it has been shown that the linear receptive field of retinal ganglion cells has a center-surround structure that closely resembles the filter predicted to maximize information about the visual scene given a constraint on the retinal output variance. Yet despite decades of investigation, the spatiotemporal properties of the linear receptive field surround have not been quantitatively assigned to interneurons. Using simultaneous intracellular and multielectrode recording we directly measured the visual feature conveyed by two parallel interneuron pathways - horizontal cells and narrow-field amacrine cells - and find that they both convey the same temporal feature at different spatial scales, acting synchronously to generate the linear receptive field surround. Moreover, we find that a linear combination of two distinct spatial scales of horizontal and narrow-field amacrine cells generates a linear receptive field that maximizes information transmission better than either horizontal or amacrine cells alone. We show that splitting the linear surround contribution into two parallel interneuron cell types allows for greater flexibility to generate an optimal surround under different environmental conditions where the signal to noise ratio can vary over many orders of magnitude. These results offer an explanation as to how and why the linear receptive field is generated in retinal ganglion cells - multiple interneuron pathways in the retina's parallel and layered circuitry make the retina efficient under a wide range of conditions.

Disclosures: L.T. McIntosh: None. **M. Manu:** None. **D.B. Kastner:** None. **B. Naecker:** None. **S.A. Baccus:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.05/P15

Topic: D.04. Vision

Support: Whitehall Foundation

Edward Mallinckrodt Jr. Foundation

Alfred P. Sloan Foundation

Research to Prevent Blindness Foundation

NIH (EY021855, EY023341, EY0268)

Title: An excitatory amacrine cell detecting object motion provides its feature-selective input to ganglion cells

Authors: *T. KIM, F. SOTO, D. KERSCHENSTEINER;
Washington Univ. In St.Louis, Saint Louis, MO

Abstract: Retinal circuits detect salient features of the visual world and report them to the brain through spike trains of retinal ganglion cells (RGCs). The most abundant RGC type in mice (W3 RGCs) selectively responds to movements of small objects, but is suppressed by global image motion caused by head, body or eye movements. Where and how object motion sensitivity arises in the retina is incompletely understood. Here, we use 2 photon guided patch clamp recordings to characterize responses of VGluT3 expressing amacrine cells (VG3 ACs) to a broad set of visual stimuli. We find that VG3 ACs are object motion sensitive and analyze the synaptic mechanisms underlying this computation. Anatomical circuit reconstructions suggest that VG3 ACs form glutamatergic synapses with W3 RGCs and targeted recordings show that the tuning of W3 RGCs' excitatory input matches that of VG3 ACs' responses. Synaptic excitation of W3 RGCs is diminished and responses to object motion are suppressed in mice lacking VGluT3. Object motion thus is first detected by VG3 ACs, which provide feature selective excitatory input to W3 RGCs. In ongoing work, we are exploring the source of surround inhibition, which suppresses responses of VG3 ACs and W3 RGCs to global image motion.

Disclosures: T. Kim: None. **F. Soto:** None. **D. Kerschensteiner:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.06/P16

Topic: D.04. Vision

Support: NIH Grant RO1EY019498

NIH Core Grant 5P30EY003176-32

Title: The development and function of direction selective circuits in the mouse retina

Authors: R. BOS, K. ZHOU, C. GAINER, *M. B. FELLER;
Univ. California, Berkeley, Berkeley, CA

Abstract: In the retina, direction-selective ganglion cells (DSGCs) respond strongly when an image is moving in the preferred direction and weakly when an image is moving in the opposite or “null” direction. Several types of DSGCs have been identified based on morphology, response properties and projections patterns. How these properties arise during development remain to be determined. We performed two-photon calcium imaging to characterize the functional organization of DSGC in developing and adult mice. Specifically, we performed recordings from ventral retina using UV drifting bars to stimulate S-opsin containing cones at different ages (P13-14, adult) and in normal and dark-reared conditions. We were able to reproducibly identify two types of DGSC (ON and ON-OFF DSGCs), verifying their categorization from responses to stationary spots. We confirmed that the directional tuning of these cells was significantly reduced in presence of a GABAA receptor antagonist or after reversibly silencing starburst amacrine cells using a pharmaco-genetic approach (Magnus et al. 2011 ; Vlasits et al, 2014). We characterized two main features of the ON and ON-OFF populations of DSGCs across development. First, we observed that directional tuning of DSGCs was established at eye-opening and remained unchanged with age or after dark-rearing. Second, we verified that in the adult, ON DSGCs clustered along three cardinal axes while ON-OFF DSGCs clustered around four, consistent with previous characterizations, However, at the time around eye opening (P13-14), the cardinal directions of both ON and ON-OFF DSGCs were diffusely distributed. Moreover, early and chronic visual deprivation prevented the maturation of the clustering around the cardinal directions. These results suggest that although the tuning of DSGCs is mature at eye-opening, their clustering along the cardinal axes is influenced by visual experience.

Disclosures: **R. Bos:** None. **K. Zhou:** None. **C. Gainer:** None. **M.B. Feller:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.07/P17

Topic: D.04. Vision

Support: NIH U01NS090562

The Glaucoma Research Foundation Catalyst for a Cure Initiative II

Neuroplasticity of Aging Training Grant 5T32AG000216-23

Title: Does the mouse retina contain 'mini-foveas' for region-specific analysis of the visual field?

Authors: *R. N. ELDANAF[1], A. D. HUBERMAN[2,3];
[1]Neurosciences, [2]Neurosciences, Neurobio. Section in the Div. of Biol. Sciences, and Ophthalmology, Univ. of California San Diego, La Jolla, CA; [3]Salk Inst. for Biol. studies, La Jolla, CA

Abstract: In many species, specific types of neurons vary their density across the retina to allow for non-uniform sampling of visual space. For example, in humans and other non-human primates, there is an increased density of certain photoreceptor and retinal ganglion cell (RGC) types in the central retina to permit higher resolution encoding of certain visual signals at that location. In recent years, the mouse has emerged as the model of choice for studying various aspects of visual system development, function and disease. It is known that there are ~30 RGC subtypes, each responsible for relaying specific visual features to the brain for central processing. It was long thought that all mouse RGC subtypes were uniformly distributed across the retina. A recent study from Wong and co-workers (Bleckert et al., Current Biology, 2014), however, showed that a specific subtype of RGCs: On-sustained alpha RGC, exhibit regional variations along the nasal-temporal axis, thereby allowing for an enhanced sampling of the binocular visual field. Motivated by those findings, we asked whether any other RGCs vary their features so as to create specialized sub-topographical maps in the mouse retina. By analyzing the size, branching, stratification and other features of transgenically labeled RGC subtypes (e.g., Huberman et al., Neuron, 2008; 2009; Osterhout et al., Neuron, 2011; Rivlin-Etzion et al., J Neurosci 2011; Dhande et al., J Neurosci, 2013) we obtained detailed analysis of how the features of individual RGC subtypes vary as a function of precise retinal location. The functional relevance of these findings for central visual processing and behavior are discussed.

Disclosures: R.N. Eldanaf: None. **A.D. Huberman:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.08/P18

Topic: D.04. Vision

Title: A novel amacrine cell circuit for signaling steady illumination in the retina

Authors: *J. JACOBY, Y. ZHU, S. H. DEVRIES, G. SCHWARTZ;
Ophthalmology, Northwestern Univ., Chicago, IL

Abstract: At the frontier of systems neuroscience is the pursuit to place identified types of neurons in the context of functional circuits. This circuit-level comprehension of neural networks is paramount in creating new therapies to combat neurodegenerative disease. The neural circuits of the retina offer unique opportunities for circuit-level analysis because of the accessibility of the tissue and our advanced knowledge of cell typology. More than sixty years of research have focused on characterizing the structure and function of most retinal cell types, but have largely left the encoding properties of retinal amacrine cells largely uncharted. Here we (i) present the first physiological recordings of a specific amacrine cell type, (ii) place this cell into a functional microcircuit, and (iii) characterize this cell's impact on the feature detection properties of its postsynaptic retinal ganglion cell. In order to fully comprehend how visual processing takes place within the retina, we must elucidate the role of amacrine cell-driven inhibitory circuits. We report that the CRH-1 amacrine cell, which was recently discovered in a transgenic mouse line, provides inhibitory drive to a new retinal ganglion cell (RGC) in mouse retina, the Suppressed-by-Contrast (SbC) RGC. Generally, retinal amacrine cells are perceived as inhibitory interneurons whose main function is modulation of visual information (through lateral inhibition, feedback control of response kinetics, balancing excitatory drive, and sharpening tuning curves). This novel neural circuit, driven by the CRH-1 amacrine cell, challenges the stereotyped class function of amacrine cells. We provide evidence that CRH-1 amacrine cell-derived feedforward inhibition can, instead, regulate the specific feature selectivity of a postsynaptic ganglion cell. Unlike typical ON, OFF, and ON-OFF cells, SbC RGCs decrease their firing in response to both light increments and decrements (i.e. activity is suppressed by high contrast). Cells with similar contrast suppression profiles have been identified in the retina of other species such as cat and rabbit, and in higher visual areas of the mouse brain. Using molecular genetic techniques, we establish direct anatomical and functional connections to the CRH-1 amacrine cell by recording in dual-cell voltage clamp from synaptically connected CRH-1 amacrine cells and SbC RGCs. This newly identified circuit reveals the mechanism of the SbC computation, in which the CRH-1 amacrine cell plays a central role. We present evidence that this amacrine cell is mediating, not simply modulating, the spiking output and feature detection of the SbC RGC.

Disclosures: **J. Jacoby:** None. **Y. Zhu:** None. **S.H. DeVries:** None. **G. Schwartz:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.09/P19

Topic: D.04. Vision

Support: NIH R01 DC012087

NIH R21 MH104756

Title: Psychophysical measurement of marmoset visual acuity as a function of eccentricity

Authors: *S. U. NUMMELA[1], C. T. MILLER[1], J. F. MITCHELL[1,2];
[1]Psychology, UCSD, LA Jolla, CA; [2]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: The common marmoset (Callithrix jacchus) is a small bodied New World monkey which offers an interesting point of comparison between mice and macaques in the study of vision (Mitchell and Leopold, 2015). However, many important facets of marmoset vision remain unknown compared to the better studied macaque monkey. Like macaques, marmosets have a specialized fovea with comparably high cone density that drops with retinal eccentricity (Troilo et al, 1993) and also show a similar cortical magnification factor in primary visual cortex (Chaplin et al, 2013). Early studies reported a peak visual acuity of 30 cycles per degree (Ordy and Samorajski, 1968) which is roughly half that of the macaque and consistent with the smaller size of their eye. One interesting feature of the marmoset retina is that beyond eccentricities of 5 degrees the cone density does not drop as rapidly as it does in macaques or humans, maintaining higher ratios of cones than rods in the periphery (Troilo et al, 1993). This could alter how behavioral measures of acuity scale as a function of eccentricity, depending on how signals are pooled from the retina and other visual areas. Therefore, we set out to confirm the initial estimates of acuity behaviorally and additionally measure how it scales as a function of retinal eccentricity. We measured visual acuity in 2 adult male marmosets at eccentricities from 1 to 10 degrees. Head-fixed marmosets were trained to initiate trials by fixating a central spot until a Gabor grating of vertical orientation appeared within one of 6 evenly spaced apertures surrounding central fixation, after which a saccade to the stimulus was rewarded with juice. Spatial frequency of the stimuli was varied using the method of constant stimuli. Like macaques, we find that acuity increases near the fovea, but at eccentricities beyond 5 degrees it remains relatively constant, roughly at 6 cycles per degree. These measurements suggest that acuity scales in proportion to the cone density at the level of the retina.

Disclosures: S.U. Nummela: None. **C.T. Miller:** None. **J.F. Mitchell:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.10/P20

Topic: D.04. Vision

Support: Foundation Fighting Blindness

NSERC

NSERC - CGS(D)

Title: Summation and division by retinal cholinergic/GABAergic starburst amacrine cells

Authors: *A. J. MCLAUGHLIN, G. AWATRAMANI;
Univ. of Victoria, Victoria, BC, Canada

Abstract: Introduction: Directionally selective ganglion cells (DSGCs) in the retina respond more robustly to moving vs. static stimuli (Barlow and Levick, 1965). GABAergic/cholinergic SACs are suggested to play a pivotal role in motion discrimination. Precisely how GABA and ACh co-modulate the DSGC's input-output function, however, remains unclear. **Methods**: Contrast response functions (CRFs) were measured for static and moving spots from superior coding DSGCs labelled in the Hb9;eGFP retina, identified using a 2-photon microscope. CRFs were quantified using the Naka-Rushton function: $R(C) = Rmax* C/(C+C_{50})$, where C_{50} is the contrast (C) that evoked a half maximal response (R). SAC activity was silenced using a DREAAD approach. **Results:** The CRF for spots moving in the DSGC's preferred direction was shifted to the left (decreased C_{50}) as well as increased in amplitude (R_{max}) compared to the CRF evoked by stationary spots. Thus motion discrimination mechanisms entail an additive (leftward shift in C_{50}) and multiplicative (increase in R_{max}) scaling of the DSGCs' stationary CRF. This effect did not appear to depend on the size of the stimulus (ranging from 50µm to 400µm in diameter). Measurement of the EPSCs and IPSCs to DSGCs suggest that motion discrimination relies on an increase in excitation and a concomitant decrease in inhibition to DSGCs. To test the involvement of GABA and ACh release from SACs in this process, we used a pharmacological approach. Blocking ACh (100 µM curare) greatly reduced motion discrimination (changes in both the C_{50} and R_{max} were reduced), while blocking GABA receptors (10 µM Gabazine, 100 µM TPMPA) enhanced it, suggesting a cross-talk between GABA and ACh signalling pathways. Interestingly, however, in the presence of GABA blockers curare selectively affected motion induced changes in C_{50}, revealing a direct additive role for ACh. Conversely, in the presence of curare, the effects of GABA blockers suggested a divisive role for GABA. Finally, to demonstrate that motion discrimination is mediated by ACh and GABA released from SACs we utilized genetic tools to silence SAC outputs to DSGCs. Preliminary results confirm a role for SACs in mediating motion discrimination. **Conclusion:** Increased ACh excitation and decreased GABA inhibition from SACs underlies the ability of DSGCs to discriminate moving from stationary objects. The results demonstrate how a single neuron perform additive and divisive operations using multiple neurotransmitter systems.

Disclosures: A.J. McLaughlin: None. **G. Awatramani:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.11/P21

Topic: D.04. Vision

Title: In which the eyes of the beholder are a worm's: Mapping of multiplexed visual cues across the array of cephalic eyes in a leech

Authors: *J. A. JELLIES[1], T. GROVES[2];
[2]Biol. Sci., [1]Western Michigan Univ., Kalamazoo, MI

Abstract: How do visual systems use simple eyes to extract information? Medicinal leeches (Hirudo) can discriminate green and near ultraviolet (UV) light (Jellies. 2014. J.Exp. Biol. 217:974) and use a distributed array of dermal sensilla as a type of "spectral statocyst" to maintain 3-D body position (Jellies. 2014. J. Comp. Physiol. A 200:923). We are extending our studies to examine how the cephalic eyes encode visual cues. Adult leeches were dissected so that the dorsal margin of the anterior sucker could be inverted, exposing the optic nerve heads of all 5 bilateral pairs of cephalic eyes for extracellular recording and light stimulation. We used previously described LEDs (red, green, blue and UV) as stimuli. Complex spiking responses from the population of about 50 photoreceptors in each eye were recorded, rectified, smoothed and integrated for comparisons. A typical response to a 2 s light pulse was a brief transient of high frequency spiking followed by a plateau of spiking. We noticed that the cephalic eyes differed in both size and apparent gaze direction. The first 2 pairs were large, elongated and directed forward, while the posterior 3 pairs were smaller, spherical and directed upward. When we examined responses across light intensity (using a series of neutral density filters), and in combinations with light adaptation, several exceptional features emerged. First, the anterior 2 pairs of eyes responded well to both green and UV light, while the posterior 3 pairs responded best to green light. Second, the anterior 2 pairs of eyes were slowly adapting, whereas the posterior 3 pairs were more rapidly adapting by several measures. The initial phasic transient was larger compared to the plateau in the posterior 3 pairs of eyes. Next, the plateau began smaller and attenuated rapidly in the posterior 3 pairs of eyes whereas the plateau showed little attenuation over 30-60 s of continuous stimulation in the anterior 2 pairs of eyes. Finally, when we stimulated eyes for 15s using a white light and superimposed LED stimuli during the white light, anterior eyes 1 and 2 showed no response. In contrast, the posterior eyes 3,4,5 responded with a strong phasic transient to the superimposed green light (and not at all to red, less so to blue and UV). Our data suggest that spectral, luminal, and temporal cues are differentially mapped across the array of cephalic eyes. The anterior, forward facing eyes might be more selectively responsive to spectral and luminal contrast, as well as shadows whereas the posterior, upward facing eyes might be more responsive to moving edges of green light. It remains to be seen how these multiplexed cues are decoded within the leech CNS to guide behavior.

Disclosures: **J.A. Jellies:** None. **T. Groves:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.12/P22

Topic: D.04. Vision

Support: Tateisi Science and Technology Foundation

Grant-in-Aid for Scientific Research 26780415

Grant-in-Aid for JSPS Fellows 13J01314

Title: Quantitative evaluation of perceptual and neuronal brightness vision of retinitis pigmentosa model rat

Authors: *N. SUEMATSU[1,2], A. SATO[3], A. KIMURA[2,4], S. SHIMEGI[2,3], S. SOMA[2,5]; [1]Grad. Sch. of Engin., [2]Grad. Sch. of Med., [3]Grad. Sch. of Frontier Biosci., Osaka Univ., Osaka, Japan; [4]Dept. of Rehabil. Sci., Osaka Hlth. Sci. Univ., Osaka, Japan; [5]Brain Sci. Inst., Tamagawa Univ., Tokyo, Japan

Abstract: Retinitis pigmentosa is a worldwide offering retinal disease, which degenerate photoreceptors and can blind the patients. Nowadays, toward cure of the disease, many studies including cell transplantation, gene therapy, neuroprotection, and retinal prosthesis have been conducted. In such research field, the Royal College of Surgeons (RCS) rat, which has impairment of phagocytic function in retinal pigment epithelium and thus photoreceptors are degenerated with age, has been used as an animal model of the disease. However, the basic perceptual and neuronal visual abilities of the RCS rat are still unclear. We previously reported that the pattern vision of RCS rat was kept until 6 weeks of age, declined gradually at 7-8 weeks of age, and finally lost after 9 weeks of age. In the current study, we focused on the brightness vision from the aspects of psychophysics and neurophysiology. We found that the RCS rats was able to complete the task related to the brightness vision at least until 11 weeks of age, but gradually became slower with weeks of age to complete the task. Moreover, neurons in the superior colliculus, the lateral geniculate nucleus, and the primary visual cortex even of the 11-week-old RCS rats exhibited enough responses to the high-luminance flash stimulus, but also the response latencies were increased with weeks of age. Our findings are sure to provide evaluation standards that are applicable to examine development of the disease and protective or restoration effects achieved by the various treatments for the disease.

Disclosures: **N. Suematsu:** None. **A. Sato:** None. **A. Kimura:** None. **S. Shimegi:** None. **S. Soma:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.13/P23

Topic: D.04. Vision

Support: Chateaubriand Fellowship

FAACTS Grant

Title: Predictive information in the retina depends on stimulus statistics

Authors: *J. M. SALISBURY[1,3], S. DENY[3], T. MORA[4], O. MARRE[3], S. PALMER[2]; [1]Univ. of Chicago, Rockton, IL; [2]Univ. of Chicago, Chicago, IL; [3]Inst. de la Vision, Paris, France; [4]École Normale Supérieure, Paris, France

Abstract: Predicting the future state of the environment is a central challenge for neural systems, both for overcoming delays due to signal transduction and for guiding future behavior. The task is so crucial that we find evidence for prediction at the sensory periphery, in the form of motion anticipation in the spiking activity of retinal ganglion cells (Berry et al., 1999; Leonardo & Meister, 2013). To quantify the retina's capacity for prediction, we use information theory to analyze ganglion cell responses in a highly simplified visual environment, consisting of a single moving bar of light. The trajectory contains both predictable and random components which set a bound on the information that the history of the stimulus contains about its future. We have previously shown that the mutual information between the present neural response and the future of the stimulus saturates this bound for one particular parametrization of the stimulus (Palmer et al., in press). Here we explore the limits of this seemingly optimal capacity for prediction by adjusting the parameters of the stimulus trajectory, making it more or less predictable. We find that adding long-range temporal correlations to the stimulus results in dramatic changes in the retina's ability to predict. When presented with a short-range correlated stimulus, the mutual information between the retinal response and the time-shifted stimulus peaks around 80 ms in the past (due to lags in signal transduction) but extends significantly into the present and near future. When we increase the range of correlations, this information extends further into the future and at higher rates because the stimulus is more predictable. Yet the retina also carries less information at its peak in the past, suggesting that it employs a different coding strategy in this statistical environment. In addition, the location of the peak shifts closer to the present, indicating that the retina is better able to compensate for sensory lags. References Berry, II, M. J., Brivanlou, I. H., Jordan, T. A. & Meister, M. (1999). Anticipation of moving stimuli by the retina. Nature, 398, 334-8. Leonardo, A., & Meister, M. (2013) Nonlinear dynamics support a linear population code in a retinal target-tracking circuit. Journal of Neuroscience, 33(43), 16971-16982. Palmer, S. E., Marre, O., Berry, II., M. J., & Bialek, W. (In press). Predictive information in a sensory population. Proceedings of the National Academy of Sciences.

Disclosures: **J.M. Salisbury:** None. **S. Deny:** None. **T. Mora:** None. **O. Marre:** None. **S. Palmer:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.14/P24

Topic: D.04. Vision

Support: DFG EXC 307

DFG BA 5283/1-1

DFG BE 5601/1-1

BMBF FKZ 01GQ1002

BW-Stiftung AZ 1.16101.09

NIH U01NS090562

Title: What the mouse´s eye tells the mouse´s brain: Novel retinal ganglion cell types

Authors: *K. FRANKE[1,2,3], T. BADEN[1,2,4], P. BERENS[1,2,4], M. ROMÁN ROSÓN[1,2,3], M. BETHGE[1,4], T. EULER[1,2,4];
[1]Werner Reichardt Ctr. For Integrative Neuroscien, Tuebingen, Germany; [2]Inst. for Ophthalmic Res., Tuebingen, Germany; [3]Grad. Sch. for Neural & Behavioural Sci., Tuebingen, Germany; [4]Bernstein Ctr. for Computat. Neurosci., Tuebingen, Germany

Abstract: In the vertebrate visual system, all output of the retina is carried by retinal ganglion cells (RGCs). Each type encodes distinct visual features in parallel for transmission to the brain. Understanding how the visual scenery is encoded by the outputs of the different RGC types will yield a complete picture of the representation of the visual scene available to the brain. Here we present a functional characterization of the retinal output channels. We show that the number of RGC types is much higher than previously thought, including many novel types of RGC. To record from every cell in the ganglion cell layer we used bulk-electroporation (Briggman & Euler, 2011) and two-photon Ca2+ imaging. A standardized stimulus set, including temporal full-field stimulation, local motion, and dense noise for receptive field mapping, was presented to the retina. Also, electrical single-cell recordings were performed to relate RGC spiking to somatic Ca2+ signals, to retrieve RGC morphologies and to characterize single cell types in more detail. We implemented a probabilistic clustering framework for separating our sample of ~9,000 cells (42 retinas) into functional clusters solely based on features extracted from their light responses using sparse PCA and mixture of Gaussians clustering. Then, the 70+ functional

clusters were post-processed into “RGC groups” based on meta data, such as immunolabels and morphological features. We found that RGCs can be divided into at least 30 functional types. These include many known cell types (OFF and ON alpha, W3, ON-OFF direction-selective), as verified using genetic label and single cell data (e.g. alpha RGCs) and additional information available (e.g. soma size/shape and retinal tiling). In addition, they include new functional types. For example, we identified an OFF DS RGC that does not co-stratify with starburst amacrine cells and an ON transient DS RGC with a single cardinal direction. Also, we found a contrast-suppressed type and a colour-opponent RGC that have not been identified in mouse before. To test if these functionally defined RGC groups correspond to single RGC types, we measured how well the dendritic fields of each type covered the retinal surface. Most RGC groups had a coverage factor (CF) of approx. 1, suggesting that they represent single types. Some groups had a CF much greater than 1, suggesting that they consist of multiple subtypes and that there may be substantially more than 30 types of RGCs in the mouse.

Disclosures: **K. Franke:** None. **T. Baden:** None. **P. Berens:** None. **M. Román Rosón:** None. **M. Bethge:** None. **T. Euler:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.15/P25

Topic: D.04. Vision

Support: NIH/NINDS (High Resolution Connectomics of Mammalian Neural Circuits, TR01 1R01NS076467-01 a

Imprinting a connectome: developmental circuit approach to mental illness, Conte 1P50MH094271-01).

Title: Specificity and intermixing in the retinogeniculate pathway of the adult mouse: a connectomic study

Authors: *J. L. MORGAN[1], A. WETZEL[3], J. W. LICHTMAN[2];
[1]MCB, [2]Mol. and cellular biology, Harvard Univ., Cambridge, MA; [3]Pittsburg Supercomputing Ctr., Pittsburgh, PA

Abstract: Serial section electron microscopy provides data for graphs of synaptic neural networks. The nodes and edges of these graphs can be mined not only for insights into the organization of neural circuits but also for histological features of individual elements. We used this dual approach on a large volume of mouse visual thalamus (400 um x 400 um x 280 um), a 100TB image dataset, to analyze the connectivity between retinal ganglion cells (RGCs) and thalamocortical neurons. The thalamocortical neurons in the dLGN of the thalamus are often

considered to be simple relays for the parallel channels of visual information generated in the retina. Consistent with this view, we found distinct types of retinal ganglion cells by their synaptic structure and found that each type tended to innervate a different subset of thalamocortical cells. However, these subsets of thalamocortical cells were intermixed both in location and connectivity. When we looked at 140 nearby thalamocortical cells we discovered many instances of convergence of different types of RGCs on the same thalamocortical cell. Moreover, anatomical markers, such as dendritic morphology, that previously had been used to identify thalamocortical cell type, were not reliable predictors of which RGCs would innervate a particular thalamocortical cell. Even though the subnetworks of neurons were not sharply delineated, we found examples of strong specificity on a more local level because individual dendrites from different neurons were innervated by the same cohort of RGC axons that hopped form one dendrite to another. Conclusion: The anatomy and network structure of RGC-to-thalamocortical cell connectivity is consistent with multiple channels of visual information passing through the same region of dLGN. The pattern of thalamocortical cell innervation by these parallel channels of retinal input is more complex than expected and at a minimum allows for considerable cross talk between retinal pathways. Furthermore, because neuronal location and shape was a poor proxy for synaptic connectivity, the standard approach, of inferring connectivity based on the appearance of a neuron, needs reexamination.

Disclosures: **J.L. Morgan:** None. **A. Wetzel:** None. **J.W. Lichtman:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.16/P26

Topic: D.04. Vision

Title: Pharmacologic dissection of neurovascular coupling pathways underlying functional retinal imaging

Authors: ***M. BEGUM**, D. TSO;
Neurosurg., SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Light-evoked reflectance decreases in the retina, seen using intrinsic signal optical imaging, are likely to be of an outer retinal origin. Other studies have shown that such reflectance changes are dominated by hemodynamics. The specific pathways linking this stimulus-driven outer retinal activity and the resulting neurovascular response (and imaged signals) are unknown. Most previous studies of retinal neurovascular coupling have been *in vitro* and presume an inner retinal origin. In this present study, we sought to dissect the role of several prospective signaling pathways in the observed stimulus-evoked neurovascular responses *in vivo* through intravitreal injections of selected agonists, antagonists and blockers. Adult cats were

anesthetized and positioned in a stereotaxic. Using a modified fundus camera, the retina was stimulated with visible (550nm) patterned stimuli and illuminated in the near-infrared (700-900nm), while intrinsic optical signals were recorded with a CCD camera. Previous retinal imaging studies using intravitreal injections of blockers of inner retinal function (e.g. TTX, PDA, APB) yielded little impact on the retinal imaging signals. In the present study, several drugs selected to interfere with a particular neurovascular coupling pathway had profound impact on the observed light-evoked retinal imaging signals. In some cases, the sign of the observed retinal imaging signal changed from negative (vasodilation) to positive (vasoconstriction). Suramin, a purinergic antagonist, initially abolished the hemodynamic response although the signal subsequently returned with a higher amplitude. With the injection of NECA, an adenosine agonist, the negative reflectance signal inverted to a positive signal. We also used inhibitors of the arachidonic acid pathway to see how its metabolites modulate the imaged response. With indomethacin, an inhibitor of prostaglandin synthase, the hemodynamic response was temporarily abolished. With PPOH, an EET synthesis inhibitor, the response disappeared for the duration of the experiment. These results indicate that arachidonic acid metabolites, and ATP metabolites play crucial roles in the light-evoked hemodynamic response. Preliminary studies of the contribution of the NO pathways via blockade of NOS with L-NAME revealed little effect on the stimulus-driven signals. These studies help establish the chain of retinal events from light absorption to observed changes in retinal reflectance *in vivo*.

Disclosures: **M. Begum:** None. **D. Tso:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.17/P27

Topic: D.04. Vision

Support: CIHR-130268-2013

Title: Differential transmission of GABA and ACh underlies directional selectivity in the mouse retina

Authors: *S. SETHURAMANUJAM, G. AWATRAMANI;
biology, Univ. of Victoria, Victoria, BC, Canada

Abstract: Introduction: In the mammalian retina, GABA/cholinergic starburst amacrine cells (SACs) play a pivotal role in specifying the response properties of directional selective ganglion cells (DSGCs). SACs provide a strong ‘null-direction’ inhibition to DSGCs, mediated through asymmetric SAC-DSGCs wiring patterns. The extent to which ACh released from SACs contributes to the DSGCs cells response and whether it stimulates the DSGC in a directional

manner is not clear. Here we sought to determine the relative contribution of nicotinic ACh receptors (nAChRs) to the DSGC's response. Methods: DSGCs were identified in a whole-mount mouse retina by their directional spiking responses measured extracellularly. Subsequently, responses to moving stimuli were measured in DSGCs voltage-clamped at a variety of different holding potentials (-70 to +40 mV). In some experiments, photoreceptor signaling was blocked (AP4 and CNQX) and responses were driven by channelrhodopsin (ChR2) expressed selectively in SACs (ChATcre-Ai32) using intense blue light (473 nm). Results:To understand the temporal dynamics of synaptic inputs to DSGCs in the physiologically intact circuit, we developed a deconvolution technique to parse out the fractional light-evoked synaptic conductances mediated by nACh, GABAA, AMPA and NMDA receptors. This was based on the assumption that the total synaptic conductance is a linear sum of the individual receptor components. We measured the voltage-dependent characteristic of each receptor type measured in pharmacological isolation, thus defining its 'basis function'. The total light-evoked synaptic conductance was then fit by the weighted sum of the individual basis functions of the different receptors. By reiterating the fitting procedure over 1 ms intervals, we extracted the temporal evolution of the different receptor mediated conductances. Our preliminary data suggests that while GABA receptor mediated conductances are large in the null direction, the nAChR component appears symmetrical. Conclusions: The results from these experiments provide for the first time an estimate of dynamic ACh signals in the DS circuit. They indicate that a differential transmission of ACh and GABA strongly contributes to the formation of directional selectivity.

Disclosures: **S. Sethuramanujam:** None. **G. Awatramani:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.18/P28

Topic: D.04. Vision

Support: NIH R01 EY024016

E. Matilda Ziegler Foundation Grant

Karl Kirchgessner Foundation Grant

Sloan Research Fellowship

Whitehall Grant

Title: Contributions of multiple synaptic mechanisms underlying direction selectivity in the retina

Authors: *W. WEI[1], Z. PEI[1], Q. CHEN[1], D. KOREN[1], B. GIAMMARINARO[2];
[1]Dept. of Neurobio., [2]Univ. of Chicago, Chicago, IL

Abstract: Direction selectivity of direction selective ganglion cells (DSGCs) in the retina results from patterned excitatory and inhibitory inputs onto DSGCs during motion stimuli. The inhibitory inputs onto DSGCs are directionally tuned to the anti-preferred (null) direction, and therefore potently suppress spiking during motion in the null direction. However, whether direction-selective inhibition is indispensable for direction selectivity is unclear. Here we selectively eliminated the directional tuning of inhibitory inputs onto DSGCs by disrupting GABA release from the presynaptic interneuron starburst amacrine cell (SAC) in the mouse retina. We found that even without directionally tuned inhibition, direction selectivity can still be implemented in a subset of On-Off DSGCs by direction selective excitation and a temporal offset between excitation and isotropic inhibition. Thus our results demonstrate the concerted action of multiple synaptic mechanisms for robust direction selectivity in the retina.

Disclosures: W. Wei: None. **Z. Pei:** None. **Q. Chen:** None. **D. Koren:** None. **B. Giammarinaro:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.19/P29

Topic: D.04. Vision

Support: NIH MH065339

Title: Characterization of medullary neuron properties in response to on and off stimuli in a locust looming detection circuit

Authors: *H. WANG[1], R. B. DEWELL[1], M. U. EHRENGRUBER[2], F. GABBIANI[1,3];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Biol., Kantonsschule Hohe Promenade, Zurich, Switzerland; [3]Computat. and Applied Mathematics, Rice Univ., Houston, TX

Abstract: In the locust visual system, the lobula giant movement detector (LGMD) and its postsynaptic target, the descending contralateral movement detector (DCMD) in the protocerebrum, are a pair of looming sensitive interneurons responding strongly to objects approaching on a collision course (looming stimuli). To this date, the neurons presynaptic to the LGMD in the medulla remain to be identified. Here, we stimulated medullary neurons expressing channelrhodopsin with laser light pulses, and recorded their spiking activities with extracellular metal electrodes and the postsynaptic response of the LGMD in the lobula with intracellular recordings. Spike sorting and correlation analysis helped to identify spontaneous medullary spike-triggered IPSPs in the LGMD. Next, moving bars and moving edges (including

both ON and OFF moving edges) were used to stimulate the same medullary neurons. Both medullary neurons and the LGMD exhibited a burst of spikes when a bar just started its motion at the border of the screen, whereas their activity was relatively weaker during the actual motion of the bar on the screen. In contrast to their response to bar movement, medullary neurons exhibited robust spiking during the movement of an OFF edge on the screen. However, the movement of an ON edge evoked relatively less spikes in the same medullary neurons. Similarly, more spikes were observed in response to OFF local visual flashes than to ON local visual flashes. Electrical stimulation close to the recording site during a looming stimulus produced IPSPs in inhibitory branch C of the LGMD, suggesting the recorded units belong to the dorsal uncrossed bundle (DUB), which is thought to mediate OFF inhibition to the LGMD. During a looming stimulus, the recorded medullary neurons exhibited an instantaneous firing rate (IFR) similar to that of the LGMD. Thus, our results indicate that these medullary neurons respond preferentially to OFF edge movement and are possibly involved in looming detection. Our results open the way for an investigation of the coding properties of OFF visual pathway medullary neurons in the context of looming detection.

Disclosures: **H. Wang:** None. **R.B. Dewell:** None. **M.U. Ehrengruber:** None. **F. Gabbiani:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.20/P30

Topic: D.04. Vision

Support: NIH RO1-EY021855

NIH RO1-EY023341

NIH P30-EY0268

NSF DGE 1143954

Title: Morphology and function of three VIP expressing amacrine cell types in the mouse retina

Authors: ***A. AKROUH**, D. KERSCHENSTEINER;
Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Amacrine cells (ACs) are the most diverse class of neurons in the retina. The variety of signals provided by ACs allows the retina to encode a wide range of visual features. Of the 30 50 AC types in mammalian species, few have been studied in detail. Here, we combine genetic and viral strategies to identify and morphologically characterize three VIP expressing GABAergic AC types (VIP1 , VIP2 and VIP3 ACs) in mice. Somata of VIP1 and VIP2 ACs

reside in the inner nuclear layer, somata of VIP3 ACs in the ganglion cell layer. Neurite arbors of VIP ACs differ in size (VIP1 ACs ≈ VIP3 ACs > VIP2 ACs) and stratify in overlapping but distinct sublaminae of the inner plexiform layer. To analyze light responses and underlying synaptic inputs, we target VIP ACs under 2 photon guidance for patch clamp recordings. VIP1 ACs depolarize strongly to light increments (ON) over a wide range of stimulus sizes, but show size selective responses to light decrements (OFF), depolarizing to small and hyperpolarizing to large stimuli. The switch in polarity of OFF responses is caused by pre and postsynaptic surround inhibition. VIP2 and VIP3 ACs both show small depolarizations to ON stimuli and large hyperpolarizations to OFF stimuli, but differ in their spatial response profiles. Depolarizations are caused by ON excitation outweighing ON inhibition, whereas hyperpolarizations result from pre and postsynaptic ON OFF crossover inhibition. Currently, we are working to map downstream circuit connections and to elucidate how VIP ACs contribute to retinal ganglion cell spike output.

Disclosures: **A. Akrouh:** None. **D. Kerschensteiner:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.21/P31

Topic: D.04. Vision

Support: NSERC 6362-2012 (MP)

RGPAS 478115-2015 (JFB)

RGPIN 2015-06582 (JFB)

194670-2014 (CC)

MOP-130337 (CC and JFB)

CIHR (JB and VH)

Title: GPR55 is involved in scotopic vision in primates

Authors: ***J. M. BOUSKILA**, V. HARRAR, C. CASANOVA, J.-F. BOUCHARD, M. PTITO; Sch. of Optometry, Univ. of Montreal, Montreal, QC, Canada

Abstract: The cannabinoid receptors CB1 (CB1R) and CB2 (CB2R) are present in the retina of many species, including mice and monkeys. We have previously reported that the G protein-coupled receptor 55 (GPR55), a putative cannabinoid receptor, is exclusively expressed in rod photoreceptors in the monkey retina, suggesting it might have a role in scotopic vision. To test

this hypothesis, we recorded full-field electroretinograms (ERGs) after the intravitreal injection of the potent and selective GPR55 antagonist, CID16020046 (CID), under light- and dark-adapted conditions. Nine vervet monkeys (Chlorocebus sabaeus) were used for this study, four controls (injected with the vehicle dimethyl sulfoxide, DMSO) and five injected with CID. We analyzed amplitudes and latencies of the a-wave and the b-wave under scotopic and photopic conditions. Our results showed that CID caused a significant reduction in the amplitude of the b-wave only in scotopic conditions (a rod-driven response). In contrast, the amplitude of the a-wave, and the latency of the a- and b-waves were not significantly different from controls following injection of CID, neither in scotopic nor in photopic conditions. These results support our anatomical findings that GPR55 is only expressed in rod photoreceptors. They also confirm the hypothesis that GPR55 plays an instrumental role in primate scotopic vision, from which we suggest clinical applications in conditions of night-blindness.

Disclosures: **J.M. Bouskila:** None. **V. Harrar:** None. **C. Casanova:** None. **J. Bouchard:** None. **M. Ptito:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.22/P32

Topic: D.04. Vision

Support: NIH NEI, R01 EY024567 (GDF)

Whitehall Foundation (GDF)

Karl Kirchgessner Foundation (GDF)

NIH, R01 EY17606 (APS)

NIH, R01 EY12155 (JC)

Title: Functional recovery in retinas of mice rescued from rod photoreceptor degeneration

Authors: ***J. P. CAFARO**[1], T. WANG[2], J. PAHLBERG[3], A. P. SAMPATH[3], J. CHEN[2], G. D. FIELD[1];
[1]Neurobio., Duke Univ., Durham, NC; [2]Zilkha Neurogenetic Institute, Univ. of Southern California, Los Angeles, CA; [3]Jules Stein Eye Inst., Los Angeles, CA

Abstract: Degeneration of rod photoreceptors, such as occurs in retinitis pigmentosa, prevents transduction of low intensity light stimuli leading to night blindness in humans. Rod degeneration also triggers degeneration of cone photoreceptors and changes downstream in the retinal circuit, eventually leading to complete blindness. Progress has been made towards

rescuing rods from cell death, but secondary changes triggered by rod degeneration may limit recovery of retinal function. Here we assess functional recovery of the retina in a novel mouse model in which photoreceptor degeneration was halted and rod functionality recovered. The mouse mutant lacks expression of the β-subunit of the cyclic nucleotide-gated channel ($CNG\beta1^{-/-}$), which can be corrected via tamoxifen-inducible Cre-mediated recombination at the endogenous locus. Rods in $CNG\beta1^{-/-}$ mice display substantially limited photoresponses and rod degeneration begins ~1 month (postnatal), with near complete loss of rods ~6 months. Tamoxifen (TM) administration restores rod photoresponses and prevents further rod cell death; thus restoring transduction, near optimally, within the limits of the degenerated retina. We assess functional recovery of the retina by measuring responses from distinct populations of retinal ganglion cells (RGCs), the retina's sole neural output, using a large-scale multi-unit electrode array. We compare RGC responses between: 1) wild type mice, 2) $CNG\beta1^{/-}$ mice without TM-induced rescue, and 3) $CNG\beta1^{-/-}$ mice with TM-induced rescue initiated at several time points during degeneration. We use dim flash responses from darkness to assess RGC threshold responses and a white noise stimulus and reverse-correlation to examine recovery of spatial and temporal receptive field properties at both rod- and cone-mediated light levels. Preliminary results show significant recovery of dim flash responses in $CNG\beta1^{-/-}$ mice when rod rescue is initiated ~1 month compared with mice without TM-induced rescue. This work provides needed insight into the limits of functional recovery in retinas that have begun photoreceptor degeneration.

Disclosures: **J.P. Cafaro:** None. **T. Wang:** None. **J. Pahlberg:** None. **A.P. Sampath:** None. **J. Chen:** None. **G.D. Field:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.23/P33

Topic: D.04. Vision

Support: NIH Grant EY15573

NSERC

Title: Synaptic signaling to rod bipolar cells in mouse retina with channelrhodopsin2-expressing horizontal cells

Authors: ***S. A. BARNES**[1], X. LIU[2], A. A. HIRANO[3], N. C. BRECHA[3];
[1]Dalhousie Univ., Halifax, NS, Canada; [2]Chongqing Univ. of Sci. and Technol., Chongqing, China; [3]UCLA, Los Angeles, CA

Abstract: Voltage-gated Ca channels in rod photoreceptors mediate the release of glutamate onto second order rod bipolar cells (BRCs) and horizontal cells (HCs). HCs send inhibitory feedback back to rods that modulates those Ca channels and may signal to rod bipolar cells (RBCs) via direct GABAergic inhibition. Inhibitory feedback has been assessed with calcium imaging in rod synaptic terminals but the response of RBCs to HC depolarization has been more challenging to detect. We expressed channelrhodopsin2 (ChR2) in Cx57-iCre HCs to investigate the effects of HC depolarization in patch clamped RBCs under light adapted conditions. Patch clamp of ChR2-expressing HCs revealed rapidly activating (τ < 1 ms) and deactivating inward currents (τ = ~23 ms) when stimulated with 10 ms steps of 480 nm light. Peak amplitude of the inward currents at saturating light intensity was 200-300 pA at -60 mV. Under current clamp, HCs depolarized rapidly in response to 50 ms, 480 nm stimulation with an overshoot, and then settled to a sustained membrane potential close to -25 mV for ~100 ms followed by a slow decay. Voltage-clamped responses of identified, Lucifer yellow-filled RBCs in retinal slices held at -80 mV during 50 ms, 480 nm stimulation showed a biphasic response having a sustained inward current (5-10 pA) lasting 200 ms followed by a transient outward rebound current (ca. 20 pA) of 200 ms time course. The inward and outward currents were both blocked by L(+)-2-amino-4-phosphonobutyrate (L-AP4). Picrotoxin blocked the rebound currents in RBCs, but since APB eliminated this current during ChR2 stimulation, this action of GABA appears to be indirect. External solutions included nipecotic acid added to reduce GABA uptake, 5 µM GABA for the degradation-synthesis cycle, pyridoxal 5'-phosphate, a GAD cofactor, and glutamine, a GABA precursor. Loss of the RBC response to 480 nm stimulation during L-AP4 block of the mGluR6-initiated cascade suggests signal transmission from rods to RBCs. If part of this signal originates in HCs and is conveyed in a picrotoxin-sensitive manner to rods, it may do so via the proposed GABAergic regulation of rod Ca channels in which the HCO_3^- permeability of HC GABARs changes synaptic cleft pH. Rod Ca channel inhibition, occurring as the result of HC GABA release and autaptic HC GABAR mediated HCO_3^- flux and cleft acidification, would reduce glutamate release during the first stage of the biphasic response, while Ca channel disinhibition could follow with a rebound release of glutamate after cleft pH recovery to a homeostatic level.

Disclosures: **S.A. Barnes:** None. **X. Liu:** None. **A.A. Hirano:** None. **N.C. Brecha:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.24/P34

Topic: D.04. Vision

Support: NIH Grant EY002576

NIH Grant EY015128

NIH Vision Core Grant EY014800

NIH Training Grant 1T32EY024234

Research to Prevent Blindness Unrestricted Award

Title: Class-specific coupling patterns among ON cone bipolar cells in the mammalian retina

Authors: *C. L. SIGULINSKY, J. S. LAURITZEN, D. P. EMRICH, C. N. RAPP, A. M. SESSIONS, R. L. PFEIFFER, K. D. RAPP, J. R. ANDERSON, R. E. MARC; Ophthalmology, Moran Eye Center, Univ. of Utah, Salt Lake City, UT

Abstract: Purpose: Gap junctions between retinal bipolar cells have been reported or predicted, but their roles, partners, and patterns remain largely unknown. Using connectomics strategies, we reconstructed the axonal arbors of 30 ON cone bipolar cells (ON CBCs) and their synaptic contacts in Retinal Connectome 1 (RC1) to map coupling topologies. Methods: RC1 is a 2 nm resolution volume of a light-adapted adult female Dutch Belted rabbit retina, built by automated transmission electron microscopy and computational assembly. ON CBCs and their coupling partners were annotated using the Viking application and explored with 3D rendering and graph visualization of connectivity. Gap junctions were validated by 0.25 nm resolution recapture with goniometric tilt as necessary. Results: Over 100 gap junctions were identified between the axonal arbors of ON CBC pairs. Three classes of ON CBCs (CBb3m, CBb4w, CBb5w) showed coupling with members of their own class (in-class coupling), producing stratified sheets of coupled cells. CBb4w and CBb5w cells formed gap junctions at each of their 7-11 (CBb5w) or 8-15 (CBb4w) contacts with neighboring class members, but their processes never overlapped, creating near perfect tiling. In contrast, CBb3m cells only made 2-4 contacts. Neighboring CBb3m arbors never overlapped, but gaps existed. Six classes of ON CBCs exhibited cross-class coupling with subsets of other ON CBC classes. Co-stratification is required for this cross-class coupling, but is not sufficient, as CBb3-4i cells coupled with CBb3m and CBb4w cells, but not with other co-stratifying CBb3 and CBb4 classes. Only CBb3m and CBb4w cells exhibited both in- and cross-class coupling, while others only cross-class coupled. CBb5w cells only in-class coupled. In contrast, CBb7 cells did not couple with any ON CBCs, but did show heterocellular coupling with AII amacrine cells. Interestingly, classes that lacked in-class coupling did not tile. Rather, they exhibited packing: suboptimal organization that lacks overlap, but results in gaps between arbors of neighboring class members. Conclusions: Gap junctions between ON CBCs are sparsely distributed, but pervasive. ON CBCs form class-specific patterns of homocellular coupling, which represent novel synaptic architectures in retinal circuitry. In- and cross-class motifs have implications in signal-to-noise control and smoothing transitions across operating ranges, respectively. The spatial correlation of in-class coupling suggests a role in defining arbor boundaries. Incorporating such coupling topologies and weights into future network models will provide more powerful predictions of network function and development.

Disclosures: C.L. Sigulinsky: None. **J.S. Lauritzen:** None. **D.P. Emrich:** None. **C.N. Rapp:** None. **A.M. Sessions:** None. **R.L. Pfeiffer:** None. **K.D. Rapp:** None. **J.R. Anderson:** None.

R.E. Marc: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signature Immunologics, Inc..

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.25/P35

Topic: D.04. Vision

Support: NIH/NEI R01 EY012141-17

Title: Information transmission at the mammalian cone to Off bipolar cell synapse

Authors: *S. DENIZ[1], C. P. RATLIFF[2], S. H. DEVRIES[1];
[1]Ophthalmology, Northwestern Univ., Chicago, IL; [2]Stein Eye Institute, UCLA, Los Angeles, CA

Abstract: In the visual system, parallel processing starts at the cone synapse when an individual cone signals to 12 or more anatomical bipolar cell types. These different bipolar cell types are distinguished by making characteristic numbers of contacts with a cone at either basal or invaginating locations. To determine how contact number and location impact the statistical properties of signaling, we recorded in voltage clamp from a presynaptic cone and a postsynaptic cone bipolar cell (cb) and measured the reproducibility of the synaptic response. Paired recordings were obtained from slices of the cone dominant ground squirrel retina. The input voltage clamp command to the cone was a 16 s white noise stimulus consistent of four 4 s repeats. The stimulus contained frequencies at equal power between 0-100 Hz with a mean of -42 to -51 mV and a standard deviation of 2.5 mV. We used the frequency-dependent bipolar cell response mean and variance to calculate the SNR and information rate for each synapse. Cone to bipolar cell pairs intracellularly labeled during recording were processed for immunohistochemistry and contact counting. We found that information rate differed between the OFF-bipolar cell types as shown in the first three columns of the table. Contacts between the recorded cells were imaged and counted using a confocal microscope. The average number of contacts for each type is listed in the next three columns.

Bipolar type	Information rate (bits.s^{-1})	± SEM	n	Contacts/cone	± SEM	n	Information rate/ cone contact (bits.s^{-1})
cb1a	181	16.5	12	1.1	0.1	10	164.5

cb1b	163.3	21.2	3	3.5	2.5	2	46.7
cb2	306.6	29.1	10	7.1	0.3	10	43.2
cb3a	249.8	24.2	17	7.2	1.2	6	34.7
cb3b	235.2	21.6	13	4.1	0.5	11	57.4

Overall, the bipolar cells that make the largest number of contacts with a cone have the highest information rates. Specifically, cb2 and cb3a cells make an average of ~7.1 contacts with a cone and have information rates of 306.6 and 249.8 bits/s^{-1}, respectively. This is in line with the general idea that more contacts enable the bipolar cell to sample release from more ribbons, which in turn provides a better estimate of the cone signal. Surprisingly, although making the fewest contacts overall, the cb1a cell had almost a 3-fold higher information rate on a per contact basis. The reasons for this increase are unknown. The results show that the cone signal undergoes a different resampling at the cone synapse in the different types of Off cone bipolar cells, and suggest a specialized function for cb1a cell contacts that remains to be determined.

Disclosures: S. Deniz: None. **C.P. Ratliff:** None. **S.H. DeVries:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.26/P36

Topic: D.04. Vision

Support: JSPS KAKENHI 25830008

Title: Contribution of presynaptic active zone proteins CAST/ELKS in the formation of retinal ribbon synapse

Authors: *A. HAGIWARA[1], Y. KITAHARA[2], C. VOGL[4], M. ABE[5], K. OHTA[3], K. NAKAMURA[3], K. SAKIMURA[5], T. MOSER[4], A. NISHI[2], T. FURUKAWA[6], T. OHTSUKA[1]; [1]Biochem., Univ. of Yamanashi, Yamanashi, Japan; [2]Pharmacol., [3]Anat., Kurume Univ. Sch. of Med., Kurume, Japan; [4]Auditory Neurosci. & InnerEarLab, Uni. Med. Ctr. Goettingen, Goettingen, Germany; [5]Brain Res. Inst., Niigata Univ., Niigata, Japan; [6]Inst. for Protein Res., Osaka Univ., Osaka, Japan

Abstract: Two types of photoreceptors - rods and cones - are specialized sensory cells that convert light into neural signals in the retina. Their unique ribbon synapses form triad synapses at a narrow band known as the outer plexiform layer (OPL) with bipolar and horizontal cells. Deletion of Bassoon and CAST, proteins of the presynaptic active zone cytomatrix, disrupts

these ribbon synapses, impairs visual function and leads to the formation of ectopic synapses of photoreceptors with bipolar and horizontal cells in the outer nuclear layer (ONL) in mutant mice. Here we explored the effect of the deletion of ELKS, a family member of CAST, and the deletion of both, CAST and ELKS, on the structure and localization of ribbon synapses as well as visual processing in retina. The ELKS gene was conditionally deleted using Cre recombinase under the control of the Crx promoter, which expresses mostly in photoreceptors. The ELKS conditional knock out (KO) mouse showed normal development, while CAST and ELKS double KO (dKO) showed serious body weight reduction. Morphological analysis of the retina indicated a drastic increase of ectopic synapses in the ONL of dKO but little synapse mislocalization in ELKS KO. Visualizing the synaptic ultrastructure by electron microscopy indicated a reduction of ribbon length by ~50% and impaired triad structure in CAST KO and dKO. Moreover, in preliminary experiments, visual function tested by scotopic electroretinogram (ERG) showed a reduction of oscillatory potentials and b-waves, suggesting visual dysfunction due to impaired transmission at ribbon synapses of dKO, while ELKS KO showed largely unaltered ERGs. From these results, we conclude that CAST and ELKS contribute to normal structure and function of retinal photoreceptor ribbon synapses.

Disclosures: **A. Hagiwara:** None. **Y. Kitahara:** None. **C. Vogl:** None. **M. Abe:** None. **K. Ohta:** None. **K. Nakamura:** None. **K. Sakimura:** None. **T. Moser:** None. **A. Nishi:** None. **T. Furukawa:** None. **T. Ohtsuka:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.27/P37

Topic: D.04. Vision

Support: NSF Grant 924372

Title: Characterization of a voltage-gated conductance and its modulation by extracellular signaling molecules in Müller cells acutely isolated from the tiger salamander retina

Authors: *B. K. TCHERNOOKOVA[1], R. P. MALCHOW[2];
[1]Biol. Sci., Univ. Of Illinois At Chicago, Chicago, IL; [2]Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: The radial glia, or Müller cells, of the vertebrate retina regulate a number of key aspects of retinal function, including providing trophic support to neurons, synthesizing neurotransmitter precursors, regulating synaptic activity through uptake of neurotransmitters, and maintaining water and ion homeostasis. In the present experiments, we examined voltage-activated currents in Müller cells acutely isolated from tiger salamander (Ambystoma tigrinum)

retinae using whole-cell voltage-clamp techniques. In the presence of 20 mM barium, we observed a voltage-dependent transient inward current that was activated by depolarization and was blocked by the addition of 200 μM cadmium. The transient inward current persisted when extracellular sodium was replaced with choline. The block by cadmium coupled with the lack of effect upon removal of extracellular sodium suggests that these currents are mediated by barium entry through voltage-dependent calcium channels. In addition, the amplitude of the inward current was significantly reduced upon superfusion of 100μM ATP. Much evidence now suggests that ATP can act as an intercellular signaling molecule in the retina and can activate purine/pyrimidine receptors on the membranes of Müller cells. Our present experiments suggest that one key effect of extracellular ATP on Müller cells is to reduce potential calcium influx through voltage-gated conductances present on these cells. We are currently using multiple techniques to investigate the ways retinal Müller cells respond to ATP and other compounds and the roles these responses may play in retinal physiology.

Disclosures: B.K. Tchernookova: None. **R.P. Malchow:** None.

Poster

330. Visual Signals in Retinal Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 330.28/P38

Topic: D.04. Vision

Support: NSF Grant 0924383

NSF Grant 0924372

Indiana Wesleyan University Hodson Summer Research Fellowship

Title: Regulation of extracellular pH by isolated Muller cells of the tiger salamander: HCO3-dependent and HCO3-independent mechanisms

Authors: *M. A. KREITZER[1], D. SWYGART[1], C. HEER[1], R. KAUFMAN[1], B. WILLIAMS[1], B. K. TCHERNOOKOVA[2], R. P. MALCHOW[2,3];
[1]Biol., Indiana Wesleyan Univ., Marion, IN; [2]Biol. Sci., [3]Ophthalmology & Visual Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: There is growing interest in the retina and the broader CNS regarding the role glial cells play in shaping synaptic transmission. In the retina, synaptic transmission is highly sensitive to small changes in extracellular pH. This has led to suggestions that extracellular pH dynamics might play an important role in shaping visual signals, including the establishment of lateral inhibition, a key feature of neuronal processing that increases perceived visual contrast. However, few studies have directly measured proton fluxes from individual identified cells. Self-

referencing H^+ selective microelectrodes have proven to be ideally suited for sensing extracellular pH changes from isolated cells. The present report builds on an ongoing characterization of regulation of retinal extracellular pH to include characterization of changes in extracellular pH mediated by Müller glial cells. We report that extracellular pH adjacent to Müller cells isolated from tiger salamander is regulated by multiple mechanisms that are Na^+ sensitive and likely contain both HCO_3^- dependent and independent components. Removal of extracellular Na^+ abolished the standing proton flux normally detected from isolated Müller cells. Amiloride, an antagonist of Na^+ dependent transport mechanisms, also significantly reduced the standing proton flux. These data support a Na^+ contribution to the regulation of extracellular pH. HCO_3^- also contributes significantly to regulation of extracellular pH. DIDS, an antagonist of HCO_3^- transport, reduced the magnitude of the standing proton extrusion from Müller cells. Surprisingly a reduction in proton flux by DIDS persisted even when HCO_3^- was not presented in the solution. This raises the possibility that DIDS may antagonize additional HCO_3^- independent processes that regulate extracellular pH, although it could also indicate contributions from endogenously produced intracellular HCO_3^-. The ability of benzolamide, an extracellular carbonic anhydrase antagonist, to enhance the magnitude of the standing proton flux lends further support for a role of HCO_3^- dependent processes in the regulation of extracellular pH by Müller cells. These findings extend previous work implicating HCO_3^- in shaping extracellular pH levels by Müller cells and suggests additional roles for HCO_3^- independent mechanisms as well. These results also warrant studies to characterize how these glial-mediated mechanisms regulating extracellular pH contribute to the processing of visual signals in the intact retina.

Disclosures: **M.A. Kreitzer:** None. **D. Swygart:** None. **C. Heer:** None. **R. Kaufman:** None. **B. Williams:** None. **B.K. Tchernookova:** None. **R.P. Malchow:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.01/P39

Topic: D.04. Vision

Support: NEI R01-EY018847

NEI P30-EY002520

NEI T32-EY07001-37

NIDA R01DA028525

DP1-OD008301

McKnight Scholar Award

Arnold \& Beckman Foundation Young Investigator Award

Title: Scaling of information in large sensory neuronal populations

Authors: *R. J. COTTON[1], A. S. ECKER[2], E. FROUDARAKIS[1], P. BERENS[2], M. BETHGE[2], P. SAGGAU[3], A. S. TOLIAS[1];
[1]Baylor Col. of Med., Houston, TX; [2]Univ. of Tuebingen, Tuebingen, Germany; [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Individual neurons are noisy. Therefore, it seems necessary to pool the activity of many neurons to obtain an accurate representation of the environment. However, it is widely believed that shared noise in the activity of nearby neurons renders such pooling ineffective, limiting the accuracy of the population code and, ultimately, behavior. However, these predictions are based on extrapolating models fit to small numbers of neurons and have not been tested experimentally. Using a novel high-speed 3D-microscope we densely recorded from hundreds of neurons in the mouse visual cortex and measured the amount of information encoded. We find that the information in this sensory population increases approximately linearly with population size and does not saturate, even for several hundred neurons. This information growth is facilitated by a correlation structure that is not aligned with the tuning, making it less harmful than would be predicted from pairwise measurements. Accordingly, a decoder that accounts for the correlation structure outperforms one that does not. Our findings suggest that sensory representations may be more accurate than previously thought and therefore that psychophysical limitations may arise from downstream neural processes rather than limitations in the sensory encoding.

Disclosures: R.J. Cotton: None. **A.S. Ecker:** None. **E. Froudarakis:** None. **P. Berens:** None. **M. Bethge:** None. **P. Saggau:** None. **A.S. Tolias:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.02/P40

Topic: D.04. Vision

Title: Characteristic temporal scales of v1 horizontal interactions

Authors: *G. BLAND[1], W. SINGER[1,2,3];
[1]Ernst Strüngmann Inst. (ESI), Frankfurt, Germany; [2]Max Planck Inst. for Brain Res., Frankfurt, Germany; [3]Frankfurt Inst. for Advanced Studies, Frankfurt, Germany

Abstract: A central tenet of our understanding of processing in V1 is the presence of feature selective neurons. Horizontal projections connect these neurons, thereby integrating visual information (review Gilbert 1992). Responses to a static bar are modulated by a flanking bar and the degree of interference depends on distance and orientation of the flanker (Kapadia et al. 1995). Activity has been observed to propagate horizontally across the visual cortex in travelling waves (review Sato et al 2012). However, our understanding of these horizontal interactions is far from complete. In this study, we measure neural responses to two visual events to assess the temporal characteristics of these interactions. To assess spatio-temporal integration in V1, we designed a modified moving bar task. Two awake monkeys passively viewed a full contrast bar moving with a particular orientation and constant orthogonal velocity while fixating. At a specific time the bar and background change to red-green isoluminance, thereby attenuating feed-forward magnocellular input. We ran this paradigm with two bar speeds (1s and 2s traversal time) and recorded unit activity and local field potentials (LFPs) using a 32 channel semi-chronic micro-drive (Grey Matter Research). As expected, the bar induced rate-increase is attenuated under isoluminant conditions relative to full contrast. The responses to the bar underwent a marked modulation when the interval between the luminance change and the passage of the bar across the receptive field was varied. With offsets between the two events >250ms the respective responses were independent. Between 250ms and 100ms, the two responses are locked at a fixed inter-response interval, dependent on the speed of the bar. When the predicted inter-response interval is <100ms, the bar induced response is completely abolished. These results demonstrate highly specific temporal interactions in V1. Visual evoked responses are independent at macro-temporal scales, stereotypic at meso-temporal scales, and strongly interfering at micro-temporal scales. At meso-temporal scales receptive field responses are shifted, with offsets that correspond to the characteristic speed of wave propagation. At micro-temporal scales the slow parvocellular input arrives in the later phase of the wave and is abolished. Thus, we support the role of a horizontal travelling wave for the purposes of predictive coding in V1.

Disclosures: **G. Bland:** None. **W. Singer:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.03/P41

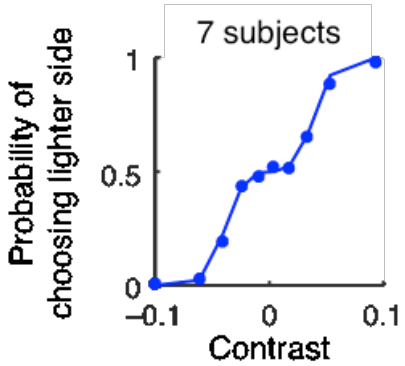
Topic: D.04. Vision

Support: ARC grant DP130102336

Title: The psychophysicist's microelectrode: weak visual stimuli reveal neuron-like response properties

Authors: *A. W. FREEMAN[1], G. LUO-LI[1], D. ALAIS[2];
[1]Univ. of Sydney, Lidcombe, Australia; [2]Univ. of Sydney, Sydney, Australia

Abstract: AIMS. 1. The visual contrast-response function in human subjects typically has low gradient at low contrast. This is inconsistent with signal detection theory, and we therefore aimed to measure in detail the contrast-response function for contrasts close to zero. 2. Recently published work shows that light decrements are detected sooner than increments. We aimed to see whether this observation held at low contrast. METHODS. Visually normal adult humans were briefly presented with horizontal grating patches. The spatial form of the gratings was a raised cosine so that each presentation provided either an increment or decrement in contrast. Either the left or right half of the grating patch was shown and subjects indicated which half had been shown. Stimuli were randomly timed, and response correctness and reaction time were recorded. RESULTS. 1. There was a small range of contrasts, centred on zero, at which contrast sensitivity was very low. The circles in the figure show mean probabilities over seven subjects. Neurons in primary visual cortex have a resting membrane potential well below threshold (Tan et al., Nature, 509, 226): we propose that the probability plateau indicates the minimum contrast required to depolarise these neurons to threshold. Indeed, incorporating an action potential threshold into standard signal detection theory fits the data well, as shown by the line in the figure. 2. Reaction times at low contrast were less for contrast decrements than for increments. This corresponds well with recently published work showing that off-dominated cortical neurons have shorter latencies to decrements than do on-dominated neurons to increments (Komban et al., Neuron, 82, 224). CONCLUSION. Low-contrast measurements reveal human behaviour that appears to reflect the properties of single neurons in primary visual cortex.



Disclosures: **A.W. Freeman:** None. **G. Luo-Li:** None. **D. Alais:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.04/P42

Topic: D.04. Vision

Support: European Union's Horizon 2020 Marie Skłodowska-Curie Grant 659593 (ProactionPerception)

European Union's FP7 Marie Curie Grant 302215 (BrainPercepts)

The Human Brain Project

Paris-Saclay Idex Icode and NeurosSaclay

CNRS

Title: Spatio-temporal synergy requirements for binding feedforward and horizontal waves in V1

Authors: *X. G. TRONCOSO, M. PANANCEAU, B. LE BEC, C. DESBOIS, F. GERARD-MERCIER, Y. FREGNAC;
Unit of Neuroscience, Information and Complexity, UNIC-CNRS, Gif sur Yvette, France

Abstract: Long distance horizontal connections, intrinsic to primary visual cortex (V1), have been hypothesized to play a role in binding cells with identical functional preferences across the visual field, irrespective of their receptive field (RF) location. Combined intracellular and imaging techniques have shown that this binding requires stimulus-induced cooperativity to enhance the long-range orientation-selective spread beyond the feedforward imprint (Chavane et al, 2011). Using test stimuli allowing spatial summation within the aggregate RF of the cortical hypercolumn, we have recently reexamined the spatio-temporal features of the synaptic subthreshold receptive field of V1 cells (Gerard-Mercier et al, SfN2014). Our results showed that synaptic responses to flashed 3-4° Gabor patches can be elicited from the far periphery (up to 15°) and, most remarkably, exhibit a coherent organization, reflecting the grouping bias of the "perceptual association field" for collinear contours (Field et al, 1993). Our new cat V1 intracellular experiments are designed to test issues critical to understand the spatial synergy and temporal coherence requirements. We used 2- and 6-stroke apparent motion (AM) concentric sequences of Gabor patches at saccadic speeds (~200°/s), centered on the subthreshold RF (with the distance between strokes scaled to the diameter of the RF) extending the motion path up to 25° into the periphery. The response to stimulation of the RF center alone was compared to the response to the AM, which was either centripetal or centrifugal with the orientation of the individual elements either collinear or cross-oriented to the motion path. Control conditions included randomized order in the AM, change in speed or contrast, ... We also included sequences restricted to the silent surround to infer filling-in responses induced by the periphery alone. Our results show a supra-linear subthreshold input from the far periphery, and a non-linear boosting of the neuronal discharge resulting in a significant phase advance (5-20 ms) in the spiking response. Summation processes during the AM sequence show de novo emergence of significant responses for stimuli flashed as far as 10-15° away from the classical RF. The boosting effect is specific to centripetal AM at saccadic speeds and could not be induced by centrifugal or random AM, or by AM at lower speeds. Collinear movement was also more effective than cross-oriented movement. All these results are consistent with our hypothesis that

cooperative “Gestalt-like” interactions are triggered when the visual input carries a sufficient level of spatial and temporal coherence matching the underlying V1 connectivity.

Disclosures: **X.G. Troncoso:** None. **M. Pananceau:** None. **B. Le Bec:** None. **C. Desbois:** None. **F. Gerard-Mercier:** None. **Y. Fregnac:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.05/Q1

Topic: D.04. Vision

Support: Welcome Trust Grant

BBSRC Grant

Title: The Helmholtz size illusion is processed by extrastriate visual cortex, evidence from TMS

Authors: ***B. D. KEEFE**[1], K. MIKELLIDOU[3], H. CLAWSON[2], A. D. GOUWS[1], P. G. THOMPSON[2], A. B. MORLAND[1];
[1]York Neuorimaging Ctr., [2]Dept. of Psychology, Univ. of York, York, United Kingdom; [3]Univ. of Pisa, Pisa, Italy

Abstract: Neuroimaging research has indicated that for some illusions of size, there are commensurate distortions of retinotopy in V1 which reduce when attention is manipulated off stimulus. It remains unclear, therefore, whether these distortions in retinotopy arise from processing within V1 or feedback from higher visual areas. To test between these possibilities we used the Helmholtz size illusion, in which physically square, horizontally lined stimuli, are perceived as taller than their physically square, vertically lined counterparts. This illusory percept can be neutralised by extending the lines to make the stimuli appear square. To explore the role of striate and extrastriate visual cortex, we performed a TMS experiment in which participants made judgements about the aspect ratio of rectangular Helmholtz stimuli that were perceptually square. We stimulated V1 and two extrastriate areas, LO1 and LO2. Only stimulation of LO1 resulted in a significant release from the illusion. Importantly, with attention maintained on stimulus during TMS, there was no significant release from the illusion during V1 stimulation. Thus we show that extrastriate, rather than primary visual cortex, plays a causal role in our perception of illusory size. Our data appear consistent with the idea that illusion related activity in V1 reflects feedback from extrastriate regions and that V1 does not necessarily play a causal role in illusory percepts.

Disclosures: **B.D. Keefe:** None. **K. Mikellidou:** None. **H. Clawson:** None. **A.D. Gouws:** None. **P.G. Thompson:** None. **A.B. Morland:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.06/Q2

Topic: D.04. Vision

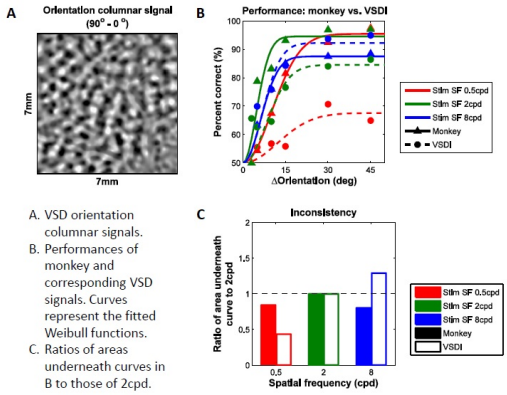
Support: NIH/NEI EY11747 for WSG

NIH/NEI EY016454 for ES

Title: Neural sensitivity in primate V1 is inconsistent with behavioral sensitivity in a fine orientation discrimination task

Authors: *Y. Y. CHEN, Y. BAI, W. S. GEISLER, E. SEIDEMANN;
Univ. of Texas at Austin, Austin, TX

Abstract: Neurons in the primate primary visual cortex (V1) are organized into columns based on their orientation preference. Although this columnar organization has been studied for decades, it is still unclear how neural activity in these columns contributes to orientation perception. As a first step towards addressing this question, we used voltage sensitive dye imaging (VSDI) to measure the columnar signals in V1 of a monkey while it performed a fine orientation discrimination task. To assess neural sensitivity, we developed a linear decoder that pools the single-trial VSDI signals over space using weights proportional to the orientation response map at the columnar scale. The decoder then uses the pooled signals to perform the same task as the monkey. If columnar signals provide the main source of information in the orientation discrimination task, the monkey's performance should be consistent with the measured neural sensitivity. To test this hypothesis, we varied the spatial frequency of the stimulus. Under the hypothesis, spatial frequency should have a similar effect on neural and behavioral sensitivities. However, we found systematic differences in the neural and behavioral effects of spatial frequency. The monkey was most sensitive at the middle frequency (2cpd), and performed worse at both low (0.5cpd) and high (8cpd) frequencies. In contrast, neural sensitivity was best at 8cpd, intermediate at 2cpd, and much worse at 0.5cpd. There are at least two possible sources that could contribute to these discrepancies between the neural and behavioral effects. First, additional sources of information beyond the columnar signals may contribute to behavioral performance at intermediate and low spatial frequencies. Second, the efficiency with which V1 signals at the columnar scale are decoded by subsequent processing stages may decrease with increasing spatial frequency. By studying trial-by-trial covariations between V1 signals and behavioral choices we may be able to distinguish between these possibilities.



A. VSD orientation columnar signals.
B. Performances of monkey and corresponding VSD signals. Curves represent the fitted Weibull functions.
C. Ratios of areas underneath curves in B to those of 2cpd.

Disclosures: **Y.Y. Chen:** None. **Y. Bai:** None. **W.S. Geisler:** None. **E. Seidemann:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.07/Q3

Topic: D.04. Vision

Support: FCT

NDSEG

NIH NEI

Title: Characterizing population-level interactions between v1 and v2

Authors: ***J. D. SEMEDO**[1], B. R. COWLEY[1], A. ZANDVAKILI[2], C. K. MACHENS[3], B. M. YU[1], A. KOHN[2];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Albert Einstein Col. of Med., New York City, NY; [3]Champalimaud Ctr. for the Unknown, Lisbon, Portugal

Abstract: Recording technology now allows us to record populations of neurons in multiple brain areas simultaneously. Traditionally, such recordings have been analyzed by identifying direct interactions between pairs of neurons, which provides a limited view of how distributed activity patterns in one area interact with those in another. We sought instead to use dimensionality reduction approaches to identify a small number of latent variables which summarize population activity patterns, and then to study the interaction of these latent variables. Specifically, we applied probabilistic canonical correlation analysis (pCCA) to populations of neurons recorded simultaneously in visual areas V1 and V2 of anesthetized macaque monkeys,

while the animals were shown sinusoidal gratings of different orientations. We used pCCA to identify response subspaces of high correlation between the populations, termed “communication subspaces”. pCCA revealed that stimulus presentation produced a robust decorrelation of V1-V2 activity, compared to the spontaneous state. To understand the basis of this effect, we examined the relationship between trial-to-trial fluctuations in V1 activity and the V1-V2 communication subspace. We found that the average angle between V1 activity and the communication subspace increases during stimulus presentation, suggesting that V1 variability is ‘filtered out’ when V2 reads out V1 activity. To account for possible delays in the communication between the two areas, we applied pCCA to time-shifted data, obtaining a multivariate cross-correlogram (mCCG) which shows the maximum correlation between the populations as a function of the time delay between them. Shortly after stimulus onset the mCCG included a clear feedforward component, with correlation being maximal for V2 responses that followed those in V1 by 2ms. Later in the evoked response, the mCCG revealed stronger correlation for V2 activity preceding V1. This suggests that the initial feedforward interaction is replaced later in the trial by a feedback interaction. Our results suggest that representing distributed activity patterns with a small number of latent variables can be revealing for understanding the interactions between distinct neuronal populations. In particular, our analysis revealed novel dynamic interactions between V1 and V2, and suggests a sophisticated communication subspace which may mitigate the detrimental effects of V1 variability on V2.

Disclosures: **J.D. Semedo:** None. **B.R. Cowley:** None. **A. Zandvakili:** None. **C.K. Machens:** None. **B.M. Yu:** None. **A. Kohn:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.08/Q4

Topic: D.04. Vision

Support: NIH grant EY016454

NIH grant EY261609

NIH grant EY11747

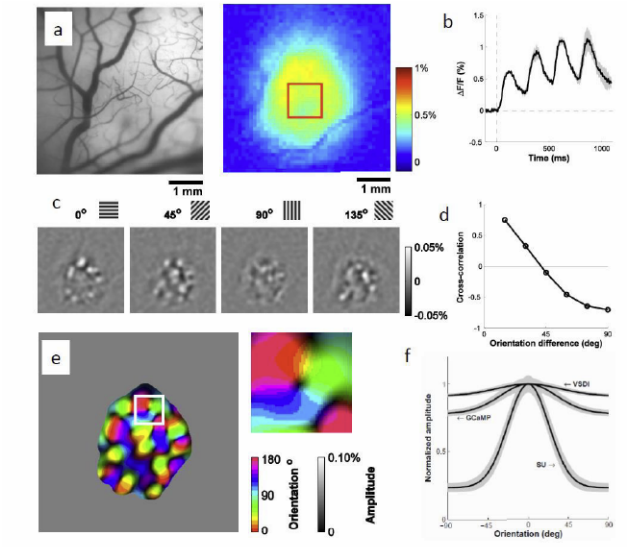
HFSP

Brain Research Foundation

Title: Long-term widefield imaging of genetically encoded calcium indicator signals in the primate visual cortex

Authors: *E. SEIDEMANN[1], Y. CHEN[2], Y. BAI[2], W. S. GEISLER[2], B. V. ZEMELMAN[2];
[1]Univ. Texas At Austin, Austin, TX; [2]Univ. of Texas at Austin, Austin, TX

Abstract: Genetically encoded calcium indicators (GECIs) can be used to measure responses from cell-type specific neural populations over large cortical regions, but until now this technique has not been adapted to awake, behaving macaque monkeys, an important model system for studying human perception, cognition and action. We used viral vectors to infect the primary visual cortex of two macaque monkeys with a transgene for a calcium indicator (GCaMP6). A few weeks after viral injection, high levels of expression of the transgene could be observed over an area of ~10mm^2 per injection site. While the monkeys performed a fixation task, calcium signals provided a robust readout of visual responses with high temporal resolution and sufficient spatial resolution to measure reliable and stable orientation maps over a period of several months. To characterize the nature of the calcium signal, we compared its tuning properties with those of a synthetic voltage sensitive dye (VSD; RH1838) across several fundamental stimulus dimensions. We found that calcium signals are more selective to stimulus orientation, less sensitive to contrast and have smaller population receptive fields than VSD signals. We then used a simple computational model to interpret these results. Our model has two components. (1) A spatial weighting function that captures the region over which neural responses are pooled to obtained the measured local imaging signal. (2) A nonlinear transfer function that describes the quantitative relation between the magnitudes of the different signals (VSD, calcium, spikes) at the single neuron level. Using this model, we find that (1) the local calcium and VSD signals reflect pooled neural activity in a Gaussian shaped region with a space constant of ~0.23 mm. (2) While VSD signals reflect membrane potentials, calcium signals are dominated by spiking activity, but also contain a small contribution from subthreshold activity. Overall our results demonstrate that chronic imaging of GECIs in behaving primates is an exciting new technique for studying the neural basis of behavior.



Calcium signals in V1 of behaving macaque. (a) Vasculature and GCaMP signal at one injection site. **(b)** Time course of GCaMP signal. **(c)** Orientation selective GCaMP signal (4 out of 12 orientations). **(d)** Pairwise correlation between maps in c. **(e)** Orientation map, **(f)** Average orientation tuning for single V1 neurons, GCaMP and VSDI signals.

Disclosures: **E. Seidemann:** None. **Y. Chen:** None. **Y. Bai:** None. **W.S. Geisler:** None. **B.V. Zemelman:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.09/Q5

Topic: D.04. Vision

Support: NIH Grant EY017605

ARO W911NF-14-1-0408

Title: Neural consequences of transcranial direct current stimulation in the primary visual cortex of awake, behaving macaques

Authors: *K. KAR[1,2], B. KREKELBERG[1];
[1]Ctr. For Mol. and Behav. Neuroscience, Rutgers Univ., Newark, NJ; [2]Behavioral and Neural Sci., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Transcranial direct current stimulation (tDCS) has been used successfully as a noninvasive neuromodulation tool to affect behavior in both the clinic and in the cognitive neurosciences. The most commonly accepted neuromodulatory effect of tDCS is a subthreshold membrane polarization. Even though some studies support the view that anodal tDCS increases cortical excitability while cathodal tDCS decreases it, reports in the literature are contradictory. To shed light on the neural consequences of tDCS, we investigated the tDCS-induced changes in neural activity in the macaque primary visual cortex. We recorded neural activity from area V1, with chronically implanted floating microelectrode arrays before and after applying tDCS. During each experimental session, we first mapped visual responses and orientation, spatial frequency, and contrast tuning using full field grating stimuli. Second, we applied tDCS for 20 minutes; either cathodal (-1 mA), anodal (+1 mA), or sham (0 mA). One (active) tDCS electrode was placed on the occipital pole of the monkey while the other (reference) was placed near the vertex. Third, we mapped the visual responses and tuning properties again, using the same stimuli. . We compared the multiunit activity (MUA) before and after stimulation. We found that 20 minutes of anodal stimulation significantly increased the multi-unit activity across multiple electrodes. This supports the view that anodal tDCS increases excitability. We did not find any specific contrast or spatial frequency dependence of this anodal tDCS-induced enhancement. Sham or cathodal tDCS did not have any consistent effect on the MUA. To our knowledge, this is the first study demonstrating the efficacy of tDCS to induce electrophysiological changes in the non-human primate. Given the significant structural and functional similarities between the macaque and human brain, and our ability to measure both behavioral and neural consequences

of transcranial stimulation, this animal model has the potential to provide unique insight into the neural mechanisms underlying tDCS and its (clinical) use in humans.

Disclosures: K. Kar: None. **B. Krekelberg:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.10/Q6

Topic: D.04. Vision

Title: Response properties and rate code of information about stimulus orientation in cat's primary visual cortex

Authors: *S. A. KOZHUKHOV, N. A. LAZAREVA;
The Inst. For Higher Nervous Activity, Moscow, Russian Federation

Abstract: It is evident that orientation tuning of neurons in primary visual cortex (V1) doesn't remain permanent during the response time course: their preferred orientation may undergo large systematic shifts. Such a phenomenon allows a single V1 cell to transmit more information about stimulus orientation that actually contains in total number of spikes by means of temporal-dependent firing rate. However the mechanism of such a process remains largely unexplored. We recorded extracellular single-unit responses of V1 cells in anesthetized and immobilized cats. The stimuli were single thin bars of different orientations. For each response we estimated spike density function to which principle component analysis was applied followed by frequency analysis based on digital filtration. We have found that each response consists of different kinds of transient and sustained components and different types of theta/alpha/beta-range oscillations. These components have different “optimal orientations” and different waveforms. The next step was to confirm that the components don't result from residuals of “neural noise”. In order do that we replaced some of them by Poisson distributed random deflections of the firing rate. As a result “surrogate responses” were built; they don't reproduce the original ones. So, we concluded that the most of components don't result from “neural noise”. Based on these data we propose the following hypothesis about how preferred orientation dynamics originates: 1. Neural response of V1 cell contains several components; each of them contains its own “optimal orientation”, unique waveform and probably its own origin. 2. Due to differences in components’ waveform different constituents may prevail in consecutive intervals of the response. 3. Change of components’ prevalence corresponds to change of preferred orientation of a stimulus during the response time course.

Disclosures: S.A. Kozhukhov: None. **N.A. Lazareva:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.11/Q7

Topic: D.04. Vision

Support: EURYI grant PE0033-117106

SNF grant 31003A_143390

University of Fribourg

Title: Effects of contrast polarity on flicker perception and primary visual cortex (V1) activity in the tree shrew

Authors: *A. KHANI, M. MOHAMED MUSTAFAR, G. RAINER;
Visual Cognition Laboratory, Inst. of Physiol., Univ. of Fribourg, Fribourg, Switzerland

Abstract: The entrainment of V1 neural activity in macaque and tree shrew exhibits marked black dominance at visual stimulation frequencies of 60Hz and above, such that light decrements elicit considerably stronger neural response transients than light increments. Here our aim is to explore the relationship between the perception of temporally modulated visual stimulation and the activity of V1 neurons. We used impulses of transient increases or decreases in luminance from a gray background at different contrasts and frequencies to generate flickering stimuli (range tested: 7.5 to 60Hz), which enabled us to study frequency-dependent sensitivity to contrast and polarity at the behavioral and neuronal level. For the behavioral study, tree shrews (Tupaia belangeri) were trained to discriminate a flickering stimulus from two other iso-luminant non-flickering stimuli in a 3 alternative forced choice (3AFC) task. We estimated the threshold contrast sensitivity from separate fits for every frequency and polarity. We observed main effects of polarity (2-way ANOVA; $F_{1, 5} = 7.02$; $p < 0.05$) and frequency ($F_{5, 25} = 4.52$; $p < 0.01$). Thus, light decrements were more easily perceived than light increments. For both polarities, performance depended on frequency, with best performance at 24Hz and 60Hz for light and dark stimuli respectively. We analyzed data from 92 V1 single neurons while flickering stimuli, identical to those used in behavioral experiments, were presented within the minimum response field of respective neurons. Preliminary analyses show that neurons exhibit a variety of response patterns, with responses depending on both frequency and polarity. Repeated measures two way ANOVAs on the firing rate of individual neurons revealed a main effect of frequency in more than half of the neurons ($n = 54$; $p < 0.05$), with peak responses typically observed at frequencies of 24 Hz ($n = 3$) and 40 Hz ($n = 51$). These analyses also showed a main effect of polarity ($n= 19$; $p < 0.05$) as well as a significant interaction ($n = 16$; $p < 0.05$). Of the 19 neurons with the main effect of polarity, only were light dominant and the overwhelming majority of those neurons were black dominant. Our findings suggest a general correspondence between black

dominance observed at the behavioral and V1 neuronal level for temporally modulated stimuli, in a frequency range from 7.5 up to 40Hz. The findings are discussed in the context of related studies in other mammalian species.

Disclosures: **A. Khani:** None. **M. Mohamed Mustafar:** None. **G. Rainer:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.12/Q8

Topic: D.04. Vision

Title: Two-photon imaging of neuronal avalanches during visual stimulation in awake mice

Authors: ***T. L. RIBEIRO**[1], S. SESHADRI[1], D. WINKOWSKI[2], P. KANOLD[2], D. PLENZ[1]; [1]Section on Critical Brain Dynamics LSN/NIMH, NIH, Bethesda, MD; [2]Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: Neuronal avalanches, measured as consecutive bouts of thresholded field potentials, are conceived to be a statistical signature that the brain operates near a critical point. In theory, criticality optimizes stimulus sensitivity, information transmission, computational capability, and mnemonic repertoires size. Recently, the cellular origin of those avalanches was demonstrated through the use of imaging techniques, revealing a power-law organization of activity in the rat brain, as the animals wake from anesthesia. It remains unclear whether the same statistical properties are present during stimulation, since the avalanche analysis is mostly restricted to ongoing activity. Moreover, the role of functionally different sub populations (e.g. barrel cortex, orientation map) in the avalanche dynamics is still unknown. In order to answer those questions we employed two-photon imaging to measure spiking activity in pyramidal cells from the primary visual cortex of mice, while they were subject to passive viewing of drifting bars of different orientation. We identified sub populations of different preferred orientation and analyzed neuronal avalanche statistics in those populations with and without visual stimulus present. Power law statistics can be identified during the periods without stimulus and deviations during stimulation were studied. The presence of a critical regime in a population of cells responsible for processing of different characteristics of a visual stimulus is an important feature for those networks.

Disclosures: **T.L. Ribeiro:** None. **S. Seshadri:** None. **D. Winkowski:** None. **P. Kanold:** None. **D. Plenz:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.13/Q9

Topic: F.04. Neuroethology

Support: JBM was supported by an ARC Australian Laureate Fellowship (FL110100103), the ARC Science of Learning Research Centre (SR120300015), and the ARC Centre of Excellence for Integrative Brain Function (CE140100007)

M.V.S. and A.Z. were supported by the Australian National Health Medical Research Council (M.V.S. APP1028210; A.Z. APP1047648)

Title: Distinct resting-state dynamics revealed by local inhibition of primary visual cortex and frontal eye fields in humans

Authors: *L. COCCHI[1], M. V. SALE[1], P. T. BELL[1], A. ZALESKY[2], L. L. GOLLO[3], M. BREAKSPEAR[3], J. B. MATTINGLEY[1];
[1]Queensland Brain Inst., Brisbane, Australia; [2]The Univ. of Melbourne, Melbourne, Australia; [3]QIMR Berghofer Med. Res. Inst., Brisbane, Australia

Abstract: Single-neuron recordings and brain imaging studies suggest that the primate cortical visual system is organized hierarchically. The primary visual cortex (area V1) mediates the flow of bottom-up visual inputs, whereas the frontal eye fields (FEF) modulate activity in V1 via top-down feedback. Interestingly, the nature of these bottom-up and top-down interactions seem to differ as a function of behavioural state. During active task performance, V1 and FEF interact dynamically in support of visual perception and selective attention. By contrast, functional magnetic resonance imaging (fMRI) studies in humans suggest that V1 and FEF are functionally segregated at rest. Here we asked whether local changes in excitability induced via transcranial magnetic stimulation (TMS) of areas V1 and FEF can alter the functional coupling between them at rest. In separate sessions, inhibitory theta-burst stimulation was applied over V1 or FEF in 21 human participants. Resting state fMRI data were obtained immediately before and after stimulation. Local frequency and whole-brain seed-to-voxel analyses revealed that larger reductions in neural activity in V1 were related to increased integration between this region and other visual areas, including FEF. Conversely, focal inhibition of FEF caused increased decoupling between this region and V1. These results suggest opposing influences of local inhibition of V1 and FEF on patterns of integration between these regions at rest. More broadly, our findings highlight the dynamic nature of hierarchical interactions in the human visual system, and provide important clues to understanding the effects of focal lesions on neural activity and behavior.

Disclosures: L. Cocchi: None. **M.V. Sale:** None. **P.T. Bell:** None. **A. Zalesky:** None. **L.L. Gollo:** None. **M. Breakspear:** None. **J.B. Mattingley:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.14/Q10

Topic: D.04. Vision

Support: EURYI grant PE0033-117106

SNF grant 31003A_143390

University of Fribourg.

Title: Decoding behavioral state from local field potential recordings in basal forebrain and visual cortex

Authors: *J. NAIR[1], A.-L. KLAASSEN[2], J. POIROT[1], A. VYSSOTSKI[3], B. RASCH[2], G. RAINER[1];
[1]Visual Cognition Laboratory, Dept. of Medicine., [2]Dept. of Psychology, Univ. of Fribourg, Fribourg, Switzerland; [3]Inst. of Neuroinformatics, Univ. of Zurich, Zurich, Switzerland

Abstract: The basal forebrain (BF) contains cholinergic and non-cholinergic neurons that project to the visual cortex (V1) where they play an important role in modulating neural network state, for example by enhancing responsivity or contrast sensitivity as well as contributing to wake/sleep regulation. Here, our aim is to explore similarities and differences between local field potential (LFP) recordings from BF and V1, in order to better understand how these brain areas function and interact during different brain states. Bilateral tungsten electrodes were implanted in the BF and V1 of rats, and LFP recordings were acquired during six hour periods from animals in their home cage using a wireless recording device. BF electrode placement was verified by BF electrical microstimulation, which could wake up animals at short latency. Based on video recordings and cortical LFP signals, the brain state was scored for 15s segments into wakefulness (W), slow-wave-sleep (SWS) and rapid-eye-movement (REM) sleep. We analyzed three LFP frequency bands: delta (1-5Hz), theta (5-10Hz) and gamma (30-80Hz). We observed considerably stronger gamma activity in BF than V1. BF gamma power was elevated during W over both SWS and REM sleep by 60% or more (paired t-tests: $p<<0.001$), whereas peak BF gamma frequency was significantly lower for W (53Hz) than both REM and SWS (60 and 58Hz respectively, $p<0.01$). We next examined how well the behavioral state could be determined using only the spectral power in pairs of frequency bands for the 15s LFP segments for each brain area. The best behavioral state classification was obtained using the delta and gamma bands in the BF (82%, vs. 77% in V1, paired t-test: $p<0.01$), whereas the other band combinations allowed performance of 76% or less with no apparent difference between V1 and BF (paired t-tests, $p>0.1$). For the theta-gamma band pair, we found that BF signals were more accurate than V1 signals in distinguishing REM from W states (7% vs. 26% errors, paired t-test:

$p<0.01$), whereas the reverse was true for distinguishing REM from SWS (25% vs. 11% errors, paired t-test: $p<0.01$). Our findings reveal substantial differences in the brain-state dependence of local neural activation patterns in basal forebrain and visual cortex. We discuss the functional implications of these findings that form the basis for functional interactions of these brain structures during sleep and wakefulness.

Disclosures: J. Nair: None. **A. Klaassen:** None. **J. Poirot:** None. **A. Vyssotski:** None. **B. Rasch:** None. **G. Rainer:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.15/Q11

Topic: D.04. Vision

Support: byNWOVICI grant 453-04-002 (to P.D.W.)

NWO VENI grant 451-09-025 (to M.R.)

Title: Spike and gamma band responses in macaque V1 and V4 to contrast modulated gratings and natural images

Authors: *M. J. ROBERTS[1,2], E. LOWET[3,2], P. DE WEERD[3,2];
[1]Univ. of Maastricht, Maastricht, Netherlands; [2]The Donders Inst. for Brain, Cognition and Behaviour, Radboud University Nijmegen, Netherlands; [3]Dept. of Neurocognition, Fac. of Psychology and Neurosci., University Maastricht, Maastricht, Netherlands

Abstract: Gamma band activity in visual cortex has been implicated in a number of cognitive and computational operations. The large majority of experiments investigating the functional significance of gamma however have used only artificial stimuli. This has led to controversy over whether findings obtained with artificial stimuli such as gratings can be extended to natural vision. This discussion is rendered difficult as studies using natural images have typically not used the same viewing conditions as for artificial stimuli, making direct comparisons impossible. We recorded multi-unit spiking and local field potentials in areas V1 and V4 of two macaque monkeys fixating centrally. We presented both gratings and natural images. Gratings of limited size with contrasts ranging between 2% and 100% were presented at or near RF locations, while natural stimuli were presented both as full screen images and as patches of a size and position comparable to the gratings. The LFP response to gratings was characterized by a strong gamma band in V1 with peak frequency dependent on stimulus contrast [1]. Gamma power was highest for intermediate contrasts. We found that responses in the gamma band to natural images on average were much weaker in both V1 and V4. This could be consistent with natural images often being poor stimuli to drive robust, synchronized neuronal responses in sufficiently large

ensembles. A recent simulation study [2] demonstrated that the magnitude of local contrast variation between stimulated cortical sites and hence local gamma frequency differences determine whether an ensemble will develop synchrony. We are currently testing whether this and other factors can predict gamma frequency and power in response to natural images. Notably, whereas gamma power on average was lower for natural images than for gratings, we found the opposite for spike rate. We also found that high contrast gratings yielded strong spiking responses and weak gamma. Ongoing analysis is anticipated to shed light on contributions of gamma to natural vision (and limits thereof), on mechanisms underlying local gamma synchronization, and on the dissociation between gamma and spiking.

Disclosures: **M.J. Roberts:** None. **E. Lowet:** None. **P. De Weerd:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.16/Q12

Topic: D.04. Vision

Support: EURYI

Title: Amplitude and frequency of visually-induced gamma-band activity are determined by age, gender and stimulus properties across a cohort of 160 human subjects

Authors: *S. VAN PELT[1,2], P. FRIES[1,3];
[1]Radboud Univ. Nijmegen, Nijmegen, Netherlands; [2]Ernst Strüngmann Inst. (ESI) for Neurosci. in Cooperation with Max Planck Society, Frankfurt, Germany; [3]Ernst Strüngmann Inst. for Neurosci. in coop. with Max Planck Society, Frankfurt, Germany

Abstract: Gamma-band synchronization has been implicated in many cortical functions, ranging from low-level visual perception to memory and attention. In recent years, visually-induced gamma-band activity has been shown to be modulated by properties of the stimulus and the task, and also by subject-dependent factors such as age and genetic make-up. In the current study, we set out to quantify the relative contributions of these elements. 160 subjects (mean age 23.2) viewed an inward moving circular sinusoidal grating while their brain activity was recorded using MEG. They had to press a button when the stimulus increased velocity. We manipulated stimulus contrast (50% and 100%), as well as stimulus velocity (0.0, 0.33, and 0.66 deg/s). We quantified the modulatory effects of stimulus properties, subject age, handedness, and gender on the peak frequency and amplitude of the induced gamma-band activity, both in isolation and combined. We also took regressors of non-interest into account such as recording date. We found that gamma-band peak frequency (for the stimulus inducing the highest signal change: 100% contrast at 0.66 deg/s) was normally distributed across the subject population, with a mean value

(+/- SD) of 56.3 +/- 5.8 Hz. Female participants had a significantly higher gamma peak frequency than men (57.0 vs 54.9 Hz). Across the entire population, peak frequency was on average 1.2 Hz lower for the 50% vs. the 100% contrast stimulus. Peak frequency decreased with 1.8 Hz per decrease in stimulus velocity of 1 deg/s. Peak frequency also decreased with increasing age, and with decreasing (age-correlated) occipital gray matter volume. Gamma amplitude showed results qualitatively similar to gamma frequency, except for an absence of gender differences. Quantitatively, amplitude effects were stronger, e.g. a 50% contrast stimulus resulted in a 60% decrease in power change relative to the 100% contrast stimulus. Multivariate, canonical correlation analysis revealed that together, stimulus contrast, stimulus velocity, subject gender and subject age explained 31% of the variance of a weighted combination of gamma peak frequency and stimulus-induced amplitude. The results indicate that there are large inter-individual differences in human gamma-band activity with frequencies spanning from 40 to 75Hz. A substantial part of variance in gamma frequency and amplitude can be accounted for by stimulus properties, and by age and gender. Studies involving gamma-band activity should take these differences into account e.g. when averaging over subjects. Future studies such as genetic association studies might further explain the remainder of the variance in gamma-band activity.

Disclosures: S. Van Pelt: None. **P. Fries:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.17/Q13

Topic: D.04. Vision

Support: NIH Grant EY022116

NIH Grant EY20485

Title: Neural synchrony and the relationship between the BOLD response and the Local Field Potential

Authors: *D. HERMES[1], M. L. NGUYEN[2], J. WINAWER[3];
[1]Stanford Univ., Stanford, CA; [2]Princeton Univ., Princeton, NJ; [3]New York Univ., New York, NY

Abstract: Purpose: Elucidating the neural circuits underlying the BOLD signal measured with fMRI is an important goal in human neuroscience. The BOLD signal has often been correlated with the local field potential (LFP), with many groups finding that BOLD correlates positively with LFP power in the gamma / high gamma band (30-150 Hz), and negatively with power in the alpha band (8-15 Hz). Individual frequency bands, however, do not correspond to distinct neural circuit responses. For example, the gamma band contains at least two distinct signals: an

oscillatory (narrowband) component and an asynchronous (broadband) component. These responses overlap in temporal frequency but differ in biological origin. We propose a model to relate BOLD and LFP in which the LFP is split into components that reflect different hypothesized neural responses rather than distinct frequency bands. Methods: Electrocorticography (ECoG) and fMRI responses to gratings and noise patterns were measured in human subjects in V1-V3. The ECoG spectral response was modeled as the sum of three components in log power/log frequency: an asynchronous broadband component (a straight line) and two narrowband oscillatory components (one Gaussian centered in the alpha band and one in the gamma band). Results: In V1, the BOLD response correlated positively and strongly with the ECoG broadband amplitude. In contrast, the BOLD response did not correlate with the ECoG gamma amplitude, even though the gamma amplitude was large and varied systematically across stimuli. In V2/V3, BOLD was again positively correlated with broadband ECoG and not with narrowband gamma. In all areas, the alpha response correlated negatively with BOLD. Next we simulated responses to a variety of stimuli in a neural population. From these synthetic neural responses, we extracted measures of BOLD and LFP based on assumptions of energetics and field summation. In the simulations, like the data, asynchronous broadband responses were highly correlated with the BOLD response, whereas the amplitude of narrowband gamma oscillations was not. Conclusion: The BOLD signal depends on the metabolic demands of a neural response, whereas LFP power depends on the level of neural synchrony and activity. The simulation provides a novel way to relate neural responses to LFP and BOLD. The amplitude of narrowband gamma oscillations does not correlate systematically with the BOLD signal because this response reflects the degree of synchrony rather than the level of neural response. Alpha oscillations negatively correlate with BOLD. Finally, broadband field potentials correlate positively with the BOLD signal, and best index the global level of neural response.

Disclosures: **D. Hermes:** None. **M.L. Nguyen:** None. **J. Winawer:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.18/Q14

Topic: D.04. Vision

Support: NIH Grant EY022116

Title: Stimulus selectivity of gamma oscillations in human visual cortex measured with magnetoencephalography

Authors: ***J. WINAWER**[1], E. R. KUPERS[1], N. CHUA[2], D. HERMES[3], K. AMANO[4];
[1]Psychology, [2]Dept. of Psychology and Ctr. for Neural Sci., New York Univ., New York, NY;

[3]Psychology, Stanford Univ., Stanford, CA; [4]Ctr. for Information and Neural Networks (CiNet), Osaka, Japan

Abstract: Background: Certain visual stimuli elicit large oscillatory cortical responses in the gamma band (30 – 80 Hz). It has been proposed that this signal plays an essential role in cortical communication and visual perception. Recent work using intracranial EEG in human showed that robust gamma oscillations can be measured for some visual stimuli (high contrast gratings) but not many other stimuli, such as noise patterns containing contrast at many orientations, constraining the possible functional role these oscillations play in vision. Here we asked (a) whether the stimulus dependence of gamma oscillations is evident in magnetoencephalography (MEG), a non-invasive measure of brain activity in healthy human subjects, and (b) whether the stimulus dependence of gamma oscillations arises from a stimulus-specific pattern of eye movements. **Methods**: During MEG recordings, subjects viewed static visual stimuli while performing a fixation color change detection task. Stimuli were viewed for either 0.5 seconds (3 subjects) or 1 s (2 subjects), with 0.5 s inter-stimulus interval (blank screen, mean luminance). Stimulus classes consisted of high contrast square wave vertical gratings spanning 0.35 to 2.90 cycles per degree, square wave plaids, or noise patterns with amplitude spectra proportional to $1/f^n$ (n=0,1,2), windowed within circular apertures subtending 24º of visual angle (diameter). Stimuli were shown in random order, with 150 repeats per class. Eye position was recorded at 1 kHz with an EyeLink 1000 eye tracker throughout the experiment. **Results**: Responses were summarized in each channel as the sum of two components, a spectrally broadband elevation over baseline (power law), and a narrowband gamma oscillation (Gaussian in log power/ log frequency, centered between 35 and 80 Hz). In all subjects, grating stimuli spanning all spatial frequencies tested elicited robust gamma oscillations (~0.5 log units above baseline), largely confined to sensors over occipital cortex. No class of noise stimuli elicited spatially specific gamma oscillations consistently across subjects. The frequency, amplitude, and direction of microsaccades did not differ systematically between viewing grating stimuli and noise stimuli. **Conclusions**: These results provide further demonstration of the strong dependence of narrowband gamma oscillations on stimulus pattern. The results also argue against the possibility that the stimulus selectivity stems from the pattern of eye movements associated with particular stimulus classes.

Disclosures: J. Winawer: None. **E.R. Kupers:** None. **N. Chua:** None. **D. Hermes:** None. **K. Amano:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.19/Q15

Topic: D.04. Vision

Support: BMBF, Bernstein Award Udo Ernst, Grant 01GQ1106

DFG priority program SPP 1665

Title: Probing communication through coherence via phase-dependent analysis

Authors: D. LISITSYN, D. HARNACK, *U. A. ERNST;
Univ. Bremen, Bremen, Germany

Abstract: Depending on behavioral context, information is selectively routed throughout the brain. Particularly in the framework of selective visual attention, neurons in V4 with multiple stimuli in their receptive fields respond as if just the attended stimulus was present [Moran, Desimone 1985]. While the mechanisms behind this effect are widely debated, communication through coherence (CTC) has emerged as a promising candidate [Fries 2005]. CTC postulates that stimulus information transmission is enhanced between oscillating populations in a favorable phase relationship, and suppressed when the oscillations are in an unfavorable or random phase relationship. Specifically, spikes from V1 arriving during peaks of V4 oscillatory activity (favorable relationship) should be much more likely to elicit further spikes in V4, resulting in effective communication; spikes arriving during troughs of V4 activity (unfavorable relationship) should fail or at least be less effective in evoking further activity. Multiple modeling and experimental studies provide support for the CTC hypothesis by showing ample evidence of attention-modulated gamma phase-locking. However, there has been no explicit research focusing on the particular temporal structure imposed on information transmission by the CTC hypothesis. In an ideal setting, the peaks in V4 should carry information from the attended stimuli (processed by V1 neurons oscillating in-phase with V4), while the unattended stimuli (processed by V1 neurons in anti-phase with V4) should only modulate activity in the troughs. In the present study, we utilize a simple, biophysically inspired model with two V1 populations having feedforward connections to a V4 population. Each V1 population is driven by a unique temporally varying stimulus, allowing us to trace the stimulus-specific information content of the activity propagated to V4 and to higher areas by utilizing spectral coherence analysis (SCA). In particular, we implement a novel form of phase-dependent SCA and quantify the contribution of the attended/non-attended signal to peaks/troughs in V4 LFPs/spikes during Gamma activity. By investigating multiple possible regimes of population activity, we find that it is possible for LFP troughs to carry information from either just the non-attended or both V1 populations. However, for information routing to succeed, the LFP peaks have to be exclusively modulated by the attended V1 population, showing the necessity of disparate phase-dependent (peak vs trough) information content. Our findings demonstrate that phase-dependent SCA can be used to directly test the CTC hypothesis in physiological recordings.

Disclosures: **D. Lisitsyn:** None. **D. Harnack:** None. **U.A. Ernst:** None.

Poster

331. Striate Cortex: Population Dynamics and Behavior.

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 331.20/Q16

Topic: D.04. Vision

Support: CNRS

The Human Brain Project

BrainScales

Paris-Saclay Idex Icode and NeurosSaclay

Title: Multiscale study of reliability and correlation of evoked cortical dynamics during natural scene processing in cat primary visual cortex

Authors: *Y. PASSARELLI, L. FOUBERT, Y. FRÉGNAC, C. MONIER;
Unit of Neuroscience, Information and Complexity, UNIC-CNRS, Gif Sur Yvette, France

Abstract: The principle of efficient coding suggests that visual processing in early sensory systems should be adapted to the statistical properties of the stimulus. By comparing intracellular responses to stimulus statistics of different complexity, we have shown (Baudot *et al.,* 2013) that the temporal reliability of the neural code is optimized for natural statistics and that the stimulus-locked trial to trial variability of the subthreshold membrane potential waveforms is modulated by the statistics of the full field stimulus context. Using the exact same stimulus seed, we present here a multiscale analysis based on more mesoscopic measures including multiple unit recordings (SUA and MUA) and local field potentials (LFP). Our aim is to explore if the single-cell observations can be related (or not) to specific behavior and stimulus dependency shared by local ensemble of neurons and if a laminar dependency of the observed effects can be detected by these mesoscopic methods and what is the global impact of input statistics changes on the correlation between neurons. In the area 17 of the anesthetized and paralyzed cat, we used Michigan silicon probes with different designs to realize laminar and lateral recordings across and within layers. To study the stimuli dependency of the reliability and correlation, we used the same stimuli as in the intracellular study, i.e, different types of visual stimuli with various statistic of increasing complexity: Drifting gratings, gratings and natural Image animated with virtual eye-movements and dense noise stimuli. To ascertain the feedforward and local vs lateral nature of the field potentials, we partitioned the full field stimulation in a central mask large enough to cover the LFP RF (equivalent to the aggregate RF of the hypercolumn) and stimuli were presented in the three center/surround partitions (center only; surround only ; center + surround). For the LFP signal, the frequency content and its reliability (measured with coherence and wavelet analysis) were highly dependent of the type of stimuli and of the layer of the recordings. Similar conclusions were obtained for spiking activity with mean rate, sparseness, Fano-factor and noise correlation measures. In particular large synchronizations of activity were found with natural image animated with saccade movement when the surround was stimulated (surround-only & center + surround).

Disclosures: **Y. Passarelli:** None. **L. Foubert:** None. **Y. Frégnac:** None. **C. Monier:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.01/Q17

Topic: D.04. Vision

Support: NIMH Intramural funding

NINDS Intramural funding

Title: Visual features driving electrophysiological responses to naturalistic movies in the marmoset ventral pathway

Authors: *J. DAY-COONEY[1], C. HUNG[2,1], B. E. RUSS[1], L. NOTARDONATO[2], A. C. SILVA[2], D. A. LEOPOLD[1];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: The visual system of the common marmoset closely resembles that of humans and Old World monkeys, including the electrophysiological responses and cytoarchitecture of the visual cortex. The marmoset's lissencephalic cortex allows for a broad sampling of neural activity across the brain's surface without interruption from gyrification. Hence, electrocorticography (ECoG) applied to the marmoset can be used to investigate spatial and temporal patterns of information simultaneously across a large portion of the visual processing hierarchy. To this end, we recorded neural responses across multiple areas in awake marmosets using subdural ECoG electrodes spaced evenly throughout the surface of the occipitotemporal cortical pathway, from primary visual cortex to rostral TE. We measured local field potential (LFP) activity as the subjects freely viewed naturalistic videos. Data from 15-minute periods of free viewing were filtered and rectified to produce a set of stimulus-driven band-limited power fluctuations at each recording site. We compared these fluctuations with the evolving features of the movie, creating a family of functional maps across the cortical surface. The strongest correlations concerned low-level movie features. Power in the beta frequency band (15 to 32 Hz) was anti-correlated to the motion content of the movies in channels spanning from early visual areas to the inferotemporal cortex, whereas power in the high-gamma band (50 to 150 Hz) was positively correlated to the contrast content with a decreasing posterior-to-anterior gradient. To further investigate the nature of the neural activity, the autocorrelation width (ACW) was calculated for each frequency band in each channel. The high-gamma band displayed an increasing posterior-to-anterior gradient for ACW sizes, whereas all other frequency bands were homogenous in ACW sizes across channels. This suggests that the temporal scale of driven high-gamma power fluctuation increased at higher stages of the visual hierarchy. To reconcile this finding with the correlational patterns calculated from the features of the movie, we used a leave-one-out method to assess the contribution of distinct time points to the observed correlations. The analysis revealed that in

early visual cortical areas, time points driving positive correlations in the gamma band coincided with those driving negative correlations in the beta band. We are currently investigating this relationship further to better understand how the beta and gamma bands of the LFP signal are driven by the content of naturalistic stimuli.

Disclosures: J. Day-Cooney: None. **C. Hung:** None. **B.E. Russ:** None. **L. Notardonato:** None. **A.C. Silva:** None. **D.A. Leopold:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.02/Q18

Topic: D.04. Vision

Support: This work was supported by a grant from the BMBF (FKZ 01GQ1002 to A.L.)

Title: Putting the pieces together - the role of parieto-occipital cortex in perceptual grouping

Authors: *K. KUTSCHEIDT[1,2,3], E. HEIN[4], M. ROTH[2,3], A. LINDNER[2];
[1]Dept. of Psychiatry and Psychotherapy, Univ. Hosp. Tübingen, Tuebingen, Germany; [2]Dept. of Cognitive Neurol., Hertie Inst. for Clin. Brain Res., Tübingen, Germany; [3]Intl. Max Planck Res. Sch., Grad. Sch. of Neural & Behavioural Sci., Tübingen, Germany; [4]Evolutionary Cognition, Univ. Tübingen/ Dept. of Psychology, Tübingen, Germany

Abstract: Our holistic perception of the environment is a result of processes, which group the fragments of information arriving at the retina in order to form entities or objects. Studies on simultanagnosia patients, who lost the ability to perceive a visual scene holistically, emphasize the importance of parieto-occipital cortex (POC) as substrate for perceptual grouping. Our aim was to further clarify the role of POC in grouping. To this end we performed an fMRI study using the Ternus display, an ambiguous apparent motion stimulus consisting of three disks presented next to each other. While two of the disks are always presented at the same position, a third disk is presented alternating between a position left and right of the two central disks. This stimulus can elicit two apparent motion percepts: element motion (EM; the outermost disk jumps back and forth across the two central stationary disks) or group motion (GM; all three disks move together as one group). It was suggested that the actual percept depends on whether and how much the Ternus elements are grouped together. The Ternus display was presented in six 5min blocks. Due to the ambiguity of the stimulus, the perceptual interpretation constantly switched between EM and GM within blocks. Participants (n=14) indicated each perceptual switch by pressing a respective button. Functional (TR=2s) and anatomical images were acquired on a 3T Siemens TRIO scanner and processed using SPM8. In participant-specific analyses we computed general linear models including three regressors: onset of GM percept, onset of EM

percept and overall stimulus presentation. For each participant, we identified task-related regions of interest (ROIs) by contrasting stimulus presentation vs. an initial 30 sec baseline. Task-related ROIs were the lingual gyrus, V3a, V5, the anterior insula, supplementary motor area, frontal eye field and the posterior intraparietal sulcus (IPS). For each individual and ROI we next extracted the time course of fMRI-activity in order to perform time-resolved group analyses identifying activity differences between EM and GM precepts. Perceptual switches were always accompanied by significant peaks in fMRI-activity in all ROIs. While the peak-amplitude did not differ between both percepts, we observed a significant shift in the temporal onset of switch-related fMRI-responses in the IPS. Specifically, there was a significantly earlier rise (about 3sec) of switch-related fMRI-activity during perceptual switches from EM to GM. This finding is consistent with the idea of a top-down influence of POC (IPS) on other areas to mediate the perception of GM by spatially grouping all individual elements to a global Gestalt.

Disclosures: **K. Kutscheidt:** None. **E. Hein:** None. **M. Roth:** None. **A. Lindner:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.03/Q19

Topic: D.04. Vision

Support: CIHR Grant 9335

Title: Visual cortical representation of word identity, font... and gender?

Authors: ***L. STROTHER**[1,2], A. M. COROS[2], Z. ZHOU[1], T. VILIS[2];
[1]Dept. of Psychology, Univ. of Nevada, Reno, Reno, NV; [2]Brain and Mind Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: We routinely encounter words in different fonts. We used fMRI to delineate neural responses to the same word viewed in different fonts from those to different words presented in the same font. Observers in our experiment viewed words under the following conditions: (a) a single word was repeated over several seconds, in the same font each time; (b) the word was repeated but the font changed for each repetition; (c) different words were presented in different fonts; (d) the font of letters in one half of a word changed; or (e) the font of a word remained constant but letters in one half of the word changed, thus changing the identity of the word each time. We observed differential lateralization in occipitotemporal cortex, including a "visual word form area" (VWFA) reported in other studies, as well as more posterior regions of occipital cortex. As expected, changes in word identity were associated with left-lateralized fMRI responses, especially in the VWFA. In contrast, changes in the font of a word while identity was preserved did not elicit strongly lateralized fMRI responses, and overlapped considerably with

left-lateralized fMRI responses to word identity changes. Our findings highlight involvement of various visual cortical areas in reading, some of which may also play a role in the rapid recognition of other types of highly familiar stimuli, such as faces. We propose to use our method to study other identity-related aspects of words, such as gender, which is relevant to both words (e.g. a person's name) and faces.

Disclosures: **L. Strother:** None. **A.M. Coros:** None. **Z. Zhou:** None. **T. Vilis:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.04/Q20

Topic: D.04. Vision

Support: Herrmann & Lilly Schilling Foundation

DFG Grant WI 406/1-1.

Title: Variability of spiking responses varies with perceptual visibility in V4, but not in pulvinar

Authors: E. POLAND[1], T. DONNER[2], D. A. LEOPOLD[3], K.-M. MÜLLER[4], *M. WILKE[5]; [1]Dept. of Cognitive Neurol., Univ. Med. Goettingen (UMG), Goettingen, Germany; [2]Dept. of Psychology, Univ. of Amsterdam, Amsterdam, Netherlands; [3]Section on Cognitive Neurophysiol. and Imaging, Natl. Inst. of Mental Health/Laboratory of Neuropsychology, Bethesda, MD; [4]The Neuromarketing Labs, Siemensstr. 3, Germany; [5]Univ. Med. Goettingen, Goettingen, Germany

Abstract: A fruitful approach to investigate the neural mechanisms of conscious perception is the employment of visual illusions that render salient visual stimuli intermittently perceptually invisible. Previous electrophysiological studies in monkeys have revealed that neuronal spiking in a wide range of brain areas correlates with subjective visibility, with a gradual increase of spike rate modulation from early to higher stages of thalamic and cortical visual processing. However, neuronal spike counts might represent only one possible mechanism how perceptual visibility is encoded by the brain. We here examined whether changes in the reported subjective visibility are associated with changes in neuronal trial-to-trial variability as assessed by the Fano factor (FF). We recorded single and multiunit spiking activity in cortical area V4 and the thalamic pulvinar in two monkeys reporting the perceptual visibility of bright luminance patches in the context of a flash suppression paradigm. Trials were sorted on the basis of reported perceptual suppression, and spiking variability was estimated using a 100 ms sliding window. To evaluate changes in Fano factor following target onset, analysis was performed on all sites independent of their visual responsiveness (V4: N = 116, Pulvinar: N = 374). For perceptual modulation analysis, we considered 33 V4 and 142 pulvinar sites that changed their firing rates

upon physical stimulus removal and evaluated significance with Wilcoxon signed-rank tests (criterion $p<0.05$). We report two main findings: 1) As expected from previous studies, the onset of the visual stimulus significantly decreased the Fano factor in area V4 (FF pre: 2.26, FF post: 1.86). Surprisingly, stimulus onset had only modest effects on variability in the pulvinar (FF pre: 1.56, FF post: 1.52). Similar results were obtained with computation of the mean-matched Fano factor. 2) Although mean firing rates in both V4 and pulvinar correlated with subjective visibility, perceptual suppression was associated with significantly reduced Fano factor only in V4. Variability of pulvinar responses was largely unmodulated by either the physical stimulus or the perceptual state. Our results suggest that sensory input or perceptual states do not stabilize neuronal responses in the pulvinar as in visual cortex. These differences in patterns of neuronal variability may support different computational roles of V4 and pulvinar in visual perception.

Disclosures: **E. Poland:** None. **T. Donner:** None. **D.A. Leopold:** None. **K. Müller:** None. **M. Wilke:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.05/R1

Topic: D.04. Vision

Support: NIH Grant EY22428

HHMI

Title: Neuronal signals supporting naturalistic texture discrimination

Authors: ***C. M. ZIEMBA**[1], R. L. T. GORIS[1,2], E. P. SIMONCELLI[1,2], J. A. MOVSHON[1]; [1]Ctr. for Neural Sci., [2]Howard Hughes Med. Inst., New York Univ., New York, NY

Abstract: The visual world is richly decorated with texture, which often serves to delineate important elements of natural scenes. In anesthetized macaque monkeys, selectivity for the key statistical features of natural texture is weak in V1, but substantial in V2, suggesting that neuronal activity in the second visual area might directly support texture perception. To test this hypothesis, we investigated the relation between single cell activity in macaque V1 and V2 and simultaneously measured psychophysical judgments of texture. We generated stimuli along a continuum between naturalistic texture (synthesized using a well-known model_Portilla & Simoncelli, 2000) and phase-randomized noise. We trained two macaque monkeys to judge whether a sample texture (presented for 500 ms in the near periphery) more closely resembled a fully naturalistic or phase-randomized texture. To enable a direct comparison of neuronal and behavioral sensitivity, we asked the monkey to discriminate the texture type (chosen from a set of five) for which a concurrently recorded neuron showed maximal selectivity. Both animals

performed the task well, with discrimination thresholds rivaling those of human observers. Under these conditions, some V1 neurons (20/72) and many V2 neurons (44/87) were tuned for naturalness. Selectivity for naturalness evolved with a time course that differed across areas. In V2, selectivity emerged early and peaked 50 ms after the initial transient. In V1, selectivity was initially absent, but increased gradually over 400 ms. Ideal observer analysis of neuronal responses revealed that single V1 and V2 neurons carried much less information about texture naturalness than the animals' behavioral reports. On average, psychophysical sensitivity exceeded neuronal sensitivity by a factor of 12 in V1 and a factor of 8 in V2. Moreover, fluctuations in the activity of single V2 neurons across repeated presentations of the same stimulus did not predict fluctuations in behavioral choice. Together these results thus suggest that texture perception arises from the combined activity of many V2 neurons.

Disclosures: C.M. Ziemba: None. **R.L.T. Goris:** None. **E.P. Simoncelli:** None. **J.A. Movshon:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.06/R2

Topic: D.04. Vision

Support: Simons foundation Grant ID# 325023

Title: Interaction between macaque face patches and border-ownership cells in response to natural face stimuli and ambiguous contours

Authors: *J. K. HESSE, P. BAO, D. Y. TSAO;
Biol., Caltech, Pasadena, CA

Abstract: Segmentation and recognition of objects in a visual scene are two interdependent problems that are hard to solve separately from each other. When trying to segment an ambiguous scene, it is helpful to already know the present objects and their shapes. However, for recognizing an object in clutter, one would like to consider its isolated segment alone to not get confounded by features of other objects. The border-ownership cells found by von der Heydt and colleagues [1] appear to play an important role in segmentation, as they are consistently selective to the side-of-figure of artificial stimuli. The present work aims to understand the interaction of border-ownership cells in retinotopic cortex and face-selective neurons in inferotemporal cortex in response to natural face stimuli. Using fMRI we first mapped retinotopy and selectivity for border-rich vs border-less stimuli to target border-ownership cells in macaque dorsal V2 and dorsal V3. Guided by this mapping we recorded simultaneously from border-ownership cells and fMRI-identified face patch MF while the monkeys passively fixated on stimuli containing faces

in isolation, overlapping faces and faces with locally ambiguous/illusory contour. We found that cells which responded consistently to the side-of-figure of artificial stimuli used by von der Heydt and others also tended to respond consistently to faces in isolation and overlapping faces. The response amplitude to faces with locally ambiguous/illusory contours was much weaker but still consistent. We are exploring the time course of information about object category in face patches and border-ownership in V2/V3 to test the hypothesis that feedback from IT might aid the formation of a surface representation of objects in retinotopic cortex, which would allow selection of that object for detailed identity recognition. 1. Zhou, Hong, Howard S. Friedman, and Rüdiger Von Der Heydt. "Coding of border ownership in monkey visual cortex." The Journal of Neuroscience 20.17 (2000): 6594-6611

Disclosures: **J.K. Hesse:** None. **P. Bao:** None. **D.Y. Tsao:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.07/R3

Topic: D.04. Vision

Support: NIH Grant EY025223

Title: Combinatorial shape logic in ventral pathway visual cortex

Authors: *S. H. SOKOL, C. E. CONNOR;
Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Categorical object recognition is remarkably robust to wide shape variations. This implies highly effective neural algorithms for abstracting consistently diagnostic combinations of shape properties. Here, we observed operations of this kind in anterior ventral pathway visual cortex of monkeys trained to discriminate letter-like shape categories in a matching task. Each category was defined by its medial axis topology (in the sense that the letter “F” is defined by a rightward L-junction at the top and a rightward T-junction near the middle). Otherwise, shapes varied widely in the lengths and widths of medial axis components, similar to variations in letter-shape across fonts. The shape categories were also mutually confusable, because they shared partial local structures. Learning a new shape category to a criterion of 85% correct performance required approximately a month of training. Following training, we analyzed neural shape coding in anterior inferotemporal cortex (AIT). A sub-fraction (70/528; 13%) of neurons in the +8-20 anterior-posterior range were significantly responsive to one or more task stimuli. To characterize shape coding precisely, we used a genetic algorithm to guide sampling of neural responses to stimuli spanning a huge domain of complex medial axis structures, varying in medial axis topology and in length, width, and orientation of medial axis components. The goal

of this procedure was to sample the response range of the neuron densely enough (with 400-1000 stimuli) to constrain a quantitative model of how shape parameters produce spike rate responses. Medial axis structures characteristic of trained stimuli emerged spontaneously from this evolutionary procedure, reflecting the effects of category discrimination learning on neural tuning in AIT. More specifically, many neurons signaled task-diagnostic logical combinations of shape fragments, representing both “and” and “not” operations. That is, their response functions combined excitatory and inhibitory tuning components that together defined consistent distinctions between trained categories. To verify the specificity of these tuning changes, we compared diagnostic value between trained stimuli and novel, untrained stimuli formed by recombining partial structures from the trained stimuli. This comparison showed that the diagnostic value of AIT response functions was specific to the trained stimuli. Our results suggest that robust object recognition depends in part on explicit, logical analysis of shape property combinations in ventral pathway visual cortex.

Disclosures: **S.H. Sokol:** None. **C.E. Connor:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.08/R4

Topic: D.04. Vision

Support: NIH RO1-EY014681

ONR N000014-14-0670

Title: Information-facilitating noise correlation in area V4 for natural images

Authors: ***S. GUAN**, R. XIA, D. SHEINBERG;
Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: The population code serves as the essential link between individual neuron’s properties and the functionality of a brain region. A fundamental question about the population code is whether and how the population differs from just a collection of independently functioning neurons. The pairwise shared noise variability of spike counts, or noise correlation, is an easily accessible and potentially powerful measure of non-independent component of population activity. Assessing the role of noise correlation in a population code is not trivial, and the literature includes multiple, often conflicting, perspectives. This is partly due to how noise correlation is experimentally quantified (Cohen & Kohn, 2011), and partly to how its impact is evaluated. In the present study, we attempted to address these discrepancies and to more objectively assess noise correlation and its role in visual coding. We used a 32-channel semi-chronic microdrive array to record spiking activity from area V4 of a macaque monkey in a

passive viewing task. Our study differed from previous investigations in four primary respects: 1) We used complex natural images as visual stimuli, instead of artificial patterns with limited variability, to better approximate signal correlation of natural vision, which may affect the role of noise correlation theoretically (Averbeck, Latham, & Pouget, 2006); 2) for every neuron pair, we quantified noise correlation independently for each stimulus rather than using the mean correlation, so as to preserve the stimulus-dependent modulation of correlation, which can be beneficial for coding (Ponce-Alvarez, Thiele, Albright, Stoner, & Deco, 2013); 3). The estimation of noise correlation was regularized with limited number of hidden factors and cross-validated, to prevent over-fitting that could lead to spurious results when evaluating the impact of noise correlation. 4) We evaluated the coding performance using the likelihood ratio and Shannon information based on a multi-variant Gaussian model, a maximum-entropy and minimal-assumption modal taking noise correlation in to account. Results from our recordings suggest that the stimulus-dependent noise correlation improves discrimination of natural stimuli in V4 population, while stimulus-averaged noise correlation does not. This provides direct evidence of an information-facilitating role of noise correlation in a naturalistic viewing situation.

Disclosures: **S. Guan:** None. **R. Xia:** None. **D. Sheinberg:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.09/R5

Topic: D.04. Vision

Support: NEI Grant EY024028

Title: Contrasting 3D shape coding strategies for objects and environments

Authors: *C. E. CONNOR[1], S. VAZIRI[2];
[1]Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD; [2]Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: We recently reported that the ventral, object-processing pathway of macaque monkey visual cortex contains some neurons that respond more strongly to large-scale, scene-like environmental stimuli (Vaziri et al. 2014, *Neuron* 84: 55-62). Here, we compared 3D shape coding of objects and environments in anterior ventral pathway. We studied individual neurons in the ventral bank of the superior temporal sulcus and the lateral convexity of the inferotemporal gyrus. We used a genetic algorithm to guide sampling of responses to abstract 3D shapes that ranged continuously from small objects to large environments extending beyond the 77º X 61º display screen. The first generation of shape stimuli was random. Successive stimulus

generations were populated by partially morphed descendants of ancestor stimuli from preceding generations. Ancestor fitness (probability of producing descendants) was a function of average neural response rate. The overall goal was comprehensive sampling of the neuron's response domain with 400-600 stimuli. To analyze neural coding, we parameterized stimulus shape in terms of 3D position, 3D surface orientation, maximum and minimum (principal) surface curvatures, and 3D orientation of minimum curvature (which captures orientation of extended edges and corners). Most neurons exhibited a strong, significant difference in responsiveness to objects vs. environments. Consistent with previous results, the shape coding range for a typical object-responsive neuron was defined by a specific configuration of mainly convex surface fragments in object-centered coordinates. In contrast, the shape coding range for a typical environment-responsive neuron comprised diverse rectilinear shape elements, linked by their orientation relationships: large planar surfaces, long exterior edges, and long interior corners, at parallel, orthogonal, and antipodal 3D orientations. Thus, these neurons integrated disparate shape cues consistent with a common 3D reference frame, typically oriented within about 30º of eye/head-centered vertical. This eclectic coding strategy could support robust perception of object/observer relationships to rooms, buildings, ground planes, and the direction of gravity.

Disclosures: C.E. Connor: None. **S. Vaziri:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.10/R6

Topic: D.04. Vision

Support: JST Presto

MIC Program

Academy of Finland

Title: Hierarchical sparse coding model and shape representation in V4

Authors: *H. HOSOYA[1], A. HYVÄRINEN[2];
[1]ATR Inst., Kyoto, Japan; [2]Dept. of Computer Sci. and HIIT, Helsinki Univ., Helsinki, Finland

Abstract: Although sparse coding theory has been successful in explaining various receptive field properties in V1, its relevance to higher visual areas has not been well studied. Previously, we investigated such relevance in V2, where we trained a three-layer sparse coding model that took outputs of standard V1 complex cell models, which in turn received natural image patches as inputs. In the resulting model, the third-layer units had excitatory fields combining local orientations that were either similar (iso-oriented excitation) or converging to a single spatial

point (convergent excitation), together with various types of inhibitory fields. We further showed that those units seemed to be related to contour, texture, or corner features in natural images and that the model reproduced three tuning properties specific to macaque V2, in a manner qualitative, quantitative, and stable across model variations. Here, we extend the previous model by adding another layer performing a similar sparse coding computation. In the extended model, the fourth-layer units were qualitatively similar to the third-layer units, representing iso-oriented or convergent excitations with various inhibition types, except that the variation in the inhibitory fields appeared to be more complicated. In addition, the model reproduced two shape tuning properties in macaque V4, again qualitatively, quantitatively, and stably across certain model variations: 1) position-specific curvature tuning reported by Pasupathy and Connor (2001) and 2) spatial invariance for straight and low-curvature-tuned units suggested by Nandy et al. (2013). Although the above model had no unit representing smooth curves unlike often suggested in prior experimental studies of V4, a variant of the model trained only with face image patches did contain such units in both third and fourth layers. Taken together, a hierarchical model that performs sparse coding of natural images in a bottom-up way produces representations that are rather simple but able to explain physiological properties not only in V2 but also in V4.

Disclosures: H. Hosoya: None. **A. Hyvärinen:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.11/R7

Topic: D.04. Vision

Support: Australian Research Council DP120103659

Title: The tuning of human visual cortex to variations in the 1/f amplitude spectra of synthetic noise images

Authors: *Z. J. ISHERWOOD[1,2], M. M. SCHIRA[3,2], B. SPEHAR[1];
[1]Sch. of Psychology, Univ. of New South Wales, Sydney, Australia; [2]Neurosci. Res. Australia, Sydney, Australia; [3]Sch. of Psychology, Univ. of Wollongong, Wollongong, Australia

Abstract: Natural scenes are characterised by a specific distribution of spatial frequencies (SF) and associated luminance intensities, known as the $1/f^{\alpha}$ amplitude spectrum. For most natural scenes, this relationship is characterised by a power function with a slope between 1.2 and 1.4 and is thought to underlie both the scale invariance and fractal properties of natural scenes. Since the visual system has evolved in a natural environment, it is theorised to be optimally tuned to such statistical regularities. However, the neural response to natural SF distributions has not been explored in great detail. Methods Functional magnetic resonance imaging (fMRI) was used to

investigate responses in early visual cortex (V1, V2, and V3) to a variety of synthetic noise images. We used a range of 1/f slopes (0.25, 0.75, 1.25, 1.75, 2.25) across two RMS contrast levels (10% and 30%) (Experiment 1) and two image types (greyscale and thresholded) (Experiment 2). Stimuli were presented in a short block design (6 seconds on with a sequence of 5 images with a 200ms ISI), across two task conditions: aesthetic rating and central visual search. The BOLD response profile was analysed using a general linear model, and the β weights were then analysed using an ANOVA. Results and Discussion The ANOVA revealed a significant main effect of slope in both experiments (01: $F_{4,11} = 29.349$, $p < 0.000$; 02: $F_{4,10} = 11.726$, $p < 0.000$). We found the BOLD response profile to resemble an inverted U-shape peaking for images with "natural" 1/f slopes (1.25) across visual areas, task conditions, contrast levels, and image types. This suggests that the visual system is tuned to statistical properties such as the natural 1/f amplitude spectrum. To gain further insight on the tuning of the visual system to the 1/f amplitude spectrum, we investigated responses as a function of eccentricity. We found a significant main effect of eccentricity in both experiments (01: $F_{4,11} = 5.083$, $p = 0.002$; 02: $F_{4,10} = 29.620$, $p < 0.000$) whereby cortical responses from the fovea to the periphery revealed the peak of the response profile to shift from the shallowest to the steepest slope, across both contrast levels, image types, and task conditions; presumably reflecting the known preference for high SFs in the fovea and low SFs in the periphery. In conclusion, we find the response profile we obtain across eccentricities (an inverted U peaking at a slope of 1.25) to be consistent with the pooling of responses across low, medium, and high SF channels, providing further insight into the neural mechanisms underlying the tuning of visual cortex to natural scene statistics such as the 1/f amplitude spectrum.

Disclosures: **Z.J. Isherwood:** None. **M.M. Schira:** None. **B. Spehar:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.12/R8

Topic: D.04. Vision

Support: National '973' Programs 2011CBA00400

China Postdoctoral Science Foundation funded project (2014M551472)

Postdoctoral Fellowship Program of Shanghai Institutes for Biological Sciences, CAS (2014KIP306)

Title: Spatial frequency selectivity in macaque V1, V2 and V4 revealed by intrinsic optical imaging

Authors: *Y. LU, H. GONG, J. YIN, Z. CHEN, I. M. ANDOLINA, W. WANG;
Inst. of Neuroscience, CAS, Shanghai, China

Abstract: One paradox in primate vision concerns the increasing complexity of encoded features while spatial frequency (SF) selectivity decreases drastically along the visual hierarchy. We know V1 holds the highest SF selectivity for fine spatial analysis, however the comparative SF processing in different functional stripes of V2 as well as in the downstream V4 is less clear. By simultaneous intrinsic optical imaging of macaque V1, V2 and V4, we quantitatively measured the population responses across these visual areas to sine-wave gratings with various SFs. As expected we found that the SF selectivity was the highest in V1 while most regions in V4 exhibited the lowest SF selectivity. The population responses of V1, V2, and V4 were subsequently confirmed by single-unit recordings in awake macaques. Compared with V1 and V4, V2 exhibited the strongest population response magnitude. We further investigated the SF selectivity across V2 functional stripes and found that the SF selectivity in thick stripes were lower than that in both thin and pale stripes. No significant SF difference was found between the thin and pale stripes. Our findings on the distinct SF selectivity across simultaneously imaged V1, V2, and V4 sheds lights on the spatial analysis along the visual hierarchy, which help to further our understanding and electrophysiological studies as well as computational modelling of the integration of local spatial features to form global representations.

Disclosures: Y. Lu: None. **H. Gong:** None. **J. Yin:** None. **Z. Chen:** None. **I.M. Andolina:** None. **W. Wang:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.13/R9

Topic: D.04. Vision

Support: NSF CRCNS Grant IIS-1309725

Title: Responses of V4 neurons to stimuli defined by inhomogeneous luminance contrast

Authors: *M. MOSHTAGH KHORASANI, W. BAIR, A. PASUPATHY;
Biol. structure, Univ. of Washington, Seattle, WA

Abstract: It is well-established that V4 neurons are sensitive to visual form. We have recently demonstrated that many V4 neurons are also sensitive to stimulus contrast polarity and can be broadly classified into four categories, Bright, Dark, Contrast and Equiluminance cells, based on their preference for the luminance contrast of shapes relative to a uniform background (Bushnell et al, 2011). Because these categories are based on homogeneous stimuli, we do not know how these cells respond to more naturalistic stimuli, where boundaries are seldom defined by a fixed

luminance contrast, and whether the different cell classes have different functional roles for encoding objects. Using a set of stimuli defined by inhomogeneous luminance contrasts, here we investigate whether preference for contrast polarity in V4 neurons is simply based on the average signed contrast along the stimulus boundary or if the contrast at key angular positions dictate responses. We also relate the spatial characteristics of contrast preference to those of form preference in single neurons to determine whether these preferences are the result of a single unified computation. We studied the responses of 54 V4 neurons in an awake fixating macaque monkey to isolated shape stimuli presented in the preferred color of the cell against an achromatic background. Local luminance contrast along the boundary of the shape was modulated by a Gaussian region of contrasting luminance that varied in size, mean luminance and position along the boundary. We studied both cases, where the Gaussian spotlight was exclusively outside or exclusively inside the stimulus boundary. Results from a 2-way ANOVA suggest that, for a majority of neurons, either just the position of the Gaussian spotlight or the position and the luminance contrast of the spotlight, has a strong influence on the responses of the neuron. Furthermore, in many neurons, the influential feature position for contrast polarity coincides with the position of the preferred shape feature. These results support the idea that the preferences for contrast polarity and shape feature along the boundary may arise from a common local computation.

Disclosures: **M. Moshtagh Khorasani:** None. **W. Bair:** None. **A. Pasupathy:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.14/R10

Topic: D.04. Vision

Support: National '973' Programs 2011CBA00400

Title: Convergent integration of various local orientations to form a global orientation from V1 and V2 to V4 in macaque

Authors: ***J. YIN**, Y. LU, Z. CHEN, H. GONG, Y. LIU, I. M. ANDOLINA, X. LI, W. WANG; Inst. of Neuroscience, CAS, Shanghai, China

Abstract: A central question in vision is how the cortex integrates local cues to form global representations along the visual hierarchy. Orientation is a key feature of a given contour and the generation of orientation selectivity offers a great window to explore the neural mechanisms underlying visual information integration. The orientation selectivity in V4 is believed to be inherited through successive convergent projections from V1 and V2 neurons with the same orientation preference. However, for a stimulus like the zebra crossing, what happens when the

global orientation and the local orientations are not matched? We investigated this question of orientation integration by simultaneous optical imaging of V1, V2, and V4 and subsequent single-unit recordings in anesthetized and awake macaques, respectively. We found that for a given orientation stimulus, the orientation domains in V1, V2, and V4 all exhibited the same orientation preference to the stimulus. However, for a global orientation stimulus defined by different local orientations, only V4 encoded the global orientation whiles V1 and V2 signaled the local orientations, suggesting V4 can integrate feed-forward inputs from V1/V2 with different orientation preferences. These population results were later confirmed by electrophysiological recordings as 85% neurons in V4, in contrast to only 28% neurons in V2 and few neurons in V1, encoded the global orientation of such stimuli. Our results demonstrate that V4 orientation domain integrates feed-forward inputs not only from V1/V2 orientation columns with the same preference, but also from those with different preferences. Our findings thus reveal the orientation integration from local into global across different visual cortices.

Disclosures: **J. Yin:** None. **Y. Lu:** None. **Z. Chen:** None. **H. Gong:** None. **Y. Liu:** None. **I.M. Andolina:** None. **X. Li:** None. **W. Wang:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.15/R11

Topic: D.04. Vision

Support: NIH Grant EY09314

Title: How do neurons in macaque visual cortex represent a high-dimensional perceptual space?

Authors: ***J. D. VICTOR**[1], Y. YU[1], D. J. THENGONE[1], J. WITZTUM[1], E. I. NITZANY[1,2], K. P. PURPURA[1];
[1]Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [2]Program in Computat. Biol. & Med., Cornell Univ., Ithaca, NY

Abstract: Perceptual spaces form the substrate for discrimination, categorization, working memory, and many other processes, and are thus a key element of mid-level vision. Color, an archetypal perceptual space, has three dimensions - but many others, such as faces and textures, have a much higher or undefined dimensionality. It is unclear how higher dimensional spaces are represented within the brain and how their representation supports further sensory processing and perceptual judgments. To probe the neural mechanisms underlying such spaces, we characterized responses of single neurons in macaque cortex to a well-characterized 10-dimensional domain of local elements of form. This space consists of black-and-white textures. Its 10 axes are determined by image statistics describing black/white balance and nearest-neighbor correlations

(Victor and Conte 2012). The space is a useful model because it captures the informative local image statistics of natural scenes (Tkačik et al., 2010). Human perceptual sensitivities are aligned to the most informative axes in the space (Hermundstad et al., 2014). Moreover, psychophysical studies suggest that this perceptual space is represented in two ways (SfN 2014): in an opponent fashion, used for detecting form near threshold, and in a distributed one, used for suprathreshold tasks. We made multi-tetrode recordings (177 sites) in V1 and V2 of 11 anesthetized and paralyzed macaques and measured neuronal tuning along and between axes in the space. Most neurons responded to multiple kinds of image statistics. Responses to 1- and 2-point statistics were common (~50%) in V1 and V2. Responses to 3- and 4-point statistics were rare (~10%) in V1, but more common (~20%) in V2, especially in the supragranular layers. Most responses indicated opponent-like tuning, but were often highly asymmetric - with a bias towards either positive or negative values of the image statistic. There was a strong bias towards sensitivity to darks in both V1 (Yeh et al., 2009, Kremkow et al., 2014) and V2. Responses were typically larger to mixtures of image statistics (i.e., along oblique directions between axes in the stimulus space) than to individual statistics (i.e., along the axes). These preferred directions varied widely across neurons, with no indication that tuning was clustered along cardinal axes. A few neurons responded in a non-monotonic fashion to variation in image statistics, suggesting that they participate in a distributed representation of the perceptual space. In sum, the study suggests that high-dimensional perceptual spaces may be represented via neurons with a wide range of preferred tunings that capture the dimensions of the spaces.

Disclosures: **J.D. Victor:** None. **Y. Yu:** None. **D.J. Thengone:** None. **J. Witztum:** None. **E.I. Nitzany:** None. **K.P. Purpura:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.16/R12

Topic: D.04. Vision

Support: German Federal Ministry of Education and Research (BMBF) grant 01GQ0420 to BCCN Freiburg

German Federal Ministry of Education and Research (BMBF) grant 01GQ0830 to BFNT Freiburg*Tübingen

DAAD (IPID4all-BrainDisC)

Title: Neural response variability in rat visual cortex

Authors: ***A. I. JASPER**[1,2], D. SUCHANEK[1,2], C. BOUCSEIN[3], A. AERTSEN[1,2], A. KUMAR[2,4];

[1]Fac. of Biology, Univ. of Freiburg, Freiburg, Germany; [2]Bernstein Ctr. Freiburg, Freiburg, Germany; [3]Multi Channel Systems MCS GmbH, Reutlingen, Germany; [4]Sch. of Computer Sci. and Communication, Royal Inst. of Technol., Stockholm, Sweden

Abstract: Noise correlations (NC) measure the trial-by-trial co-variability of the spiking activities of two neurons in response to a repeatedly presented stimulus. Previous studies have shown that the strength of these noise correlations depends on several factors, like the distance between the recorded neurons, the similarity of their tuning properties, and the local circuitry (1). Furthermore, NC in layer 4 of the primary visual cortex (V1) of macaques were shown to be significantly lower than in layers 2/3 or 5/6 (2,3). Macaques, and also cats used in similar studies, have a visual cortex with highly structured functional maps, resulting in neighbouring neurons sharing similar tuning properties. Furthermore, neurons with similar preferred orientations but located in different pinwheels are connected via long-range patchy connections. By contrast, rats have a visual cortex without such functional maps and it also lacks long-range patchy connections (4). It is, therefore, unclear whether NC in rats show similar properties as reported for species with structured cortices. Correlations in spontaneous activity in the rat visual cortex were shown to depend on the difference in preferred orientation, rather than on the distance between neurons (5). We further investigated this question by recording spontaneous and visual stimulus-evoked spiking activity in V1 of anaesthetized rats. We found that in layer 2/3 the NC in stimulus-evoked activity are significantly lower than in spontaneous activity ($p<0.002$, Mann-Whitney-test, Bonferroni corrected), whereas layer 4 showed no difference. We also found that NC in layer 2/3 were significantly higher than in layer 4 in both stimulus-evoked and spontaneous activity ($p<0.03$ and $p<0.0001$, same tests). Finally, we found that NC during evoked activity in layer 2/3 decreased with increasing difference in preferred orientation ($p< 0.01$, Kruskal-Wallis-test), whereas no dependency was found in layer 4. Our findings indicate that the neural response variability in rat visual cortex resembles that in species with structured functional maps and long-range patchy connections, suggesting that these features do not play a major role in shaping the neural response variability. References 1 Cohen MR, Kohn A (2011) Nat Neurosci 14 (7): 811-819 2 Hansen BJ et al. (2012) Neuron 76(3):590 - 602. 3 Smith MA, Sommer MA (2013) J Neurosci. 33(12):5422-5432 4 van Hooser SD et al. (2006) J Neurosci, 26(29):7680-7692. 5 Ch'ng YH, Reid, RC (2010) Front Integr Neurosci, 4.

Disclosures: **A.I. Jasper:** None. **D. Suchanek:** None. **C. Boucsein:** None. **A. Aertsen:** None. **A. Kumar:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.17/R13

Topic: D.04. Vision

Support: NIH R01 EY024912

P50 MH103204

NIH P30 EY08098

NIH P41RR03631

Title: Divisive normalization in monkey inferotemoral cortex is biased in favor of familiar images

Authors: *T. MEYER, S. RAMACHANDRAN, C. R. OLSON;
CNBC, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Images rendered familiar through repeated passive viewing over many days elicit weak neuronal responses in monkey inferotemporal cortex. Weakening arises from the fact that the population response, although it begins at normal strength, is sharply truncated at around 100 ms. In the experiment described here, we set out to determine whether weakening of the response is accompanied by a disadvantage in the competition for neuronal representation under conditions in which a familiar image and a novel image are presented simultaneously. We first gave the monkey prolonged exposure to 16 images of objects. While the monkey directed gaze at a central fixation spot, the 4°x 4° image was presented at an eccentricity of 4.6° in either the upper or lower visual field contralateral to the hemisphere destined for recording. Eight of the images were always presented at the upper location and the other eight were always presented at the lower location. Over the course of two months, the monkey experienced each image 830 times. During recording at each site, we selected for use two images that had been presented in the same quadrant during familiarization training and two session-specific novel images. The familiar images were presented only in the trained quadrant and the novel images only in the complementary quadrant. On interleaved trials, we recorded responses to each of the four images presented as a singleton and to each of the four combinations obtained by pairing a familiar image with a novel image. Each display lasted 600 ms. Upon analyzing data from 31 neurons in one monkey, we found that familiar and novel images elicited highly distinctive responses. The responses to familiar images in particular took the form of an oscillation phase-locked to stimulus onset at a frequency of around 5 Hz. To determine whether a neuron preferentially represented the familiar or the novel image during simultaneous presentation, we first determined which familiar image and which novel image elicited stronger firing during singleton displays. Then, using a best-minus-worst measure, we assessed the strength of each representation. We found that familiar images were represented markedly more strongly than novel images. We conclude that familiar images, although they elicit weak responses when presented alone, actually exhibit a competitive advantage relative to simultaneously presented novel images.

Disclosures: T. Meyer: None. **S. Ramachandran:** None. **C.R. Olson:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.18/R14

Topic: D.04. Vision

Support: EY9314

Title: An approach to the study of mid-level vision in the alert macaque monkey

Authors: *K. P. PURPURA, J. L. BAKER, J.-W. RYOU, J. D. VICTOR;
Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Early visual cortex in primates transforms the activity in the retinogeniculate pathway into signals that higher cortical areas use for making inferences about the surfaces, objects, and movements in the local environment that guides much of behavior. A complete description of the nature of these transformations at the mid-level of the visual cortical hierarchy (V1, V2, V3 and V4: V1-4) remains a central goal of visual neuroscience. V1-4 are well-defined neuroanatomical modules, each with distinct neurophysiological properties, yet these regions are also tightly interconnected and are nearly simultaneously active following the appearance of an image on the retina. Thus, to carry out an adequate investigation of mid-level vision in non-human primates it is necessary to collect neurophysiological signals, at both the single-unit and local population level, simultaneously from V1-4 and to do so in the context of visually-guided behavior. It also necessary to utilize sets of visual stimuli that will allow for the testing of hypotheses about the computations that underlie the transformations implemented in V1-4. We have developed an approach to the study of mid-level vision that can meet both challenges. To obtain recordings simultaneously from V1-4 we implanted a 32-microelectrode microdrive (GMR, Gray Matter Research Bozeman MT) above the lunate sulcus of a macaque monkey. Due to the folding of the cortex in and around the lunate, microelectrode tracks directed from the dorsal surface, from within an area several millimeters in diameter centered on the sulcus, are able to sample multiple sites across V1-V4 (Gattass et al. 1988). The geometry of the sulcus places many of the sites of interest at a range of depths with respect to the cortical surface, which the GMR allows us to access. We used a set of visual stimuli that provide a general framework for investigating the neural processing across V1-4. The stimuli consist of black-and-white textures that are drawn from a domain specified by image statistics that determine black/white balance and nearest-neighbor correlations (Victor and Conte 2012). These stimuli have been well-characterized psychophysically, capture informative aspects of natural scenes (Hermundstad et al 2014) and have used been used recently to determine how V1 and V2 contribute to the encoding of local elements of form and shape (Yu et al. 2015). Our preliminary studies with the approach described here show that stable, and well-isolated multi-area neural recordings can be obtained over months from V1-4, and that both inter-regional interactions and local activity carry signatures for stages of processing in mid-level vision.

Disclosures: **K.P. Purpura:** None. **J.L. Baker:** None. **J. Ryou:** None. **J.D. Victor:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.19/R15

Topic: D.04. Vision

Title: Spatially extended models of visual cortical networks with balanced excitation and inhibition

Authors: ***C. EBSCH**;
Univ. of Notre Dame, South Bend, IN

Abstract: In the primary visual cortex (V1), synaptic connection probabilities depend on location and functionality [1]. Connectivity structure in V1 is well-studied, but the relationship between between connectivity, network dynamics and visual coding remains an open problem. A clue to understanding visual cortical coding comes from the observation that V1 circuits operate in an inhibitory-stabilized or balanced regime in which strong recurrent excitation is balanced by strong inhibition [2,3]. We study the implications of balanced excitation and inhibition on visual coding using spatially extended models of visual cortical circuits. We extend the mean-field theory of balanced networks to account for arbitrary spatial structure. We find that, in the balanced state, firing rate profiles are determined by the solution to a linear Fredholm integral equation. Modeling the spatial structure of V1 circuits reveals that excitatory-inhibitory balance promotes the sharpening of orientation tuning curves and produces surround-suppression dynamics even when inhibitory neurons project more locally than excitatory neurons. We supplement our mean field analysis with simulations of integrate-and-fire network with biologically realistic parameters. [1] Bosking, W. H., Zhang, Y., Schofield, B., & Fitzpatrick, D. (1997). Orientation selectivity and the arrangement of horizontal connections in tree shrew striate cortex. The Journal of Neuroscience 17(6), 2112–27. [2] Van Vreeswijk, C., & Sompolinsky, H. (1996). Chaos in neuronal networks with balanced excitatory and inhibitory activity. Science, 274(5293), 1724–6. [3] Ozeki, H., Finn, I. M., Schaffer, E. S., Miller, K. D., & Ferster, D. (2009). Inhibitory stabilization of the cortical network underlies visual surround suppression. Neuron, 62(4), 578–92.

Disclosures: **C. Ebsch:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.20/R16

Topic: D.04. Vision

Support: IRP of NINDS

IRP of NIMH

Title: Common modules of visual responses to naturalistic movies across macaque and marmoset an fmri study

Authors: *C.-C. HUNG[1], B. E. RUSS[2], J. R. DAY-COONEY[2], C. C. YEN[3], R. A. BERMAN[2], L. NOTARDONATO[3], A. C. SILVA[3], D. A. LEOPOLD[2];
[1]NINDS, NIH, Gaithersburg, MD; [2]NIMH, [3]NINDS, NIH, Bethesda, MD

Abstract: The ventral visual pathway of the primate brain supports important aspects of high-level vision, including those pertaining to social perception. Establishing the evolution of functionally defined subareas of the ventral pathway, as well as their homology across different primate species, is of great value toward understanding the organizational principles of the visual brain. The specialization for particular visual stimuli in ventral stream visual areas has been studied in great detail, generally using flashed static images or short clip of movement sequences. Recently, studies involving free viewing of extended, naturalistic videos have begun to complement this approach and reveal additional aspects of the brain's functional organization (Russ and Leopold, 2015). As it is straightforward to present the same video content to a wide range of experimental subjects, this set of methods has opened new doors for comparison of functional specialization across species (Mantini et al., 2012). Previously, we presented static images to the common marmoset to demonstrate the existence of multiple face-selective patches that bore a strong resemblance to those known to exist in the macaque (Hung et al., 2015). The present study investigates possible homological correspondence among these and other functionally defined visual areas based on whole-brain fMRI collected from both species watching the same set of videos. We analyzed the results from three perspectives. First, feature-based mapping revealed a broadly similar pattern of functional maps that were driven by video features such as image contrast, motion energy, and face content. Second, following Mantini et al., we created function maps based solely on the shared voxel time courses using independent component analysis (ICA). While these maps were generated without the knowledge of the movie contents in a data-driven way, they largely recapitulated the feature maps and suggest several parallel information processing stream along the ventral pathway. Finally, we used the relative weighting (loading) of the independent components to interrogate specific functional subregions, essentially generating a fingerprint of coefficients for a given voxel or region of interest. Initial results using this method suggest that individual face patches within each species have different fingerprints, with unique relative contributions of IC's associated with non-face attributes, such as motion energy and image contrast. These methods may be able to establish

putative correspondences between the specific face patches in marmosets, macaques, and humans.

Disclosures: **C. Hung:** None. **B.E. Russ:** None. **J.R. Day-Cooney:** None. **C.C. yen:** None. **R.A. Berman:** None. **L. Notardonato:** None. **A.C. Silva:** None. **D.A. Leopold:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.21/R17

Topic: D.04. Vision

Support: CIHR grant MOP-14825

Title: Spatiotemporal properties of neurons in the ventral stream of the cat visual cortex

Authors: *B. O. SOUZA[1], C. CASANOVA[2];
[1]École d'Optométrie, École D'Optométrie, Univ. De Montréal, Montreal, QC, Canada; [2]Ecole d'Optometrie, Ecole D'Optometrie, Univ. De Montreal, Montreal, QC, Canada

Abstract: The visual cortex is organised in a hierarchical manner in which higher cortical areas integrate different inputs in order to process complex visual features. Area 21 in the cat is considered as a homolog of area V4 in primates and is known to play an important role in form detection. While responses of area 21a neurons have been described in previous studies, there is little information, if any, about the spatiotemporal properties of the receptive fields (RFs) of cells in this cortical area. The aim of the present study was to determine the spatiotemporal profiles of 21a neurons. Visual stimuli were displayed on a screen subtending 110 x 90 deg of visual field. A white noise stimulus was used to characterize the spatio-temporal characteristics of area 21a receptive fields and consisted in a pseudo-randomized sequence of bright and dark squares (4x4 deg) briefly presented (35 ms) against a gray background. Full field drifting sinusoidal gratings with varying directions, spatial and temporal frequencies were also used to assess neuronal basic properties. First-order spatiotemporal profiles of 50 neurons were obtained. For most neurons, bright subfields were larger than dark ones (239.95 ± 26.5 vs 141.39 ± 13.2 deg2, Student's t test, $p<0.001$) and, for most cells (45/50), subfields were overlapping (33 ± 2% overlap), The average maximal spike probability of bright subfields was greater than that for dark subfields (0.043 ± 0.0061 vs 0.022 ± 0.0032, $p<0.001$). In the time domain, the activity of most subfields (45/50) overlapped but the bright subfields'activity peaked earlier than that of their dark counterpart (61.42 ± 8.68 vs 87.04 ± 12.3 ms, $p<0.001$). The spatio-temporal profiles of area 21a neurons receptive fields are considerably different from those described in the cat motion cortical area (i.e. the posteromedial part of the lateral suprasylvian cortex), supporting a distinct role of this cortical area in visual processing.

Disclosures: **B.O. Souza:** None. **C. Casanova:** None.

Poster

332. Extrastriate Cortex: Representing Objects and Texture

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 332.22/R18

Topic: D.04. Vision

Support: NWO VENI 451.13.023

NWO VICI 400.09.198

NWO Brain & Cognition 433.09.208

7th Framework Program PITN-GA-2011-290011

ERC Grant Agreement n. 339490

Title: Bottom-up and top-down priorities modulate responses in macaque visual cortex

Authors: ***P. C. KLINK**[1], J. A. M. LORTEIJE[2], B. VAN VUGT[1], P. R. ROELFSEMA[1]; [1]Netherlands Inst. For Neurosci., Amsterdam, Netherlands; [2]Fac. of Science, Swammerdam Inst. for Life Sci., Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Goal-directed behavior requires selection of an appropriate response from among a broader repertoire of potential actions. Priority signals like bottom-up stimulus salience and top-down reward expectation influence this response selection process. While bottom-up saliency is often regarded a built-in feature of the visual system, active suppression of salient distractors has been reported in parietal cortex after extensive training (Ipata, Gee, Gottlied, Bisley, & Goldberg, 2006). Here we investigate the neural signature of bottom-up and top-down priorities for color and shape in macaque visual cortical area V4. We recorded multi-unit activity from two monkeys while they performed a visual search task in which they identified the odd-shape-out, either a circle among five squares or a square among five circles, by making an eye movement towards it. All stimulus shapes had the same color, red or green, except for one of the non-targets. This color singleton was drawn in the other color, making it a pop-out salient distractor. Animals were randomly rewarded with a large or small juice reward for correct target selection and stimulus color schemes were randomly switched on subsequent trials. On correct trials, neuronal responses to target shapes in the receptive field were enhanced relative to non-targets, whereas responses to salient distractors were suppressed. This priority-based response modulation was apparent approximately 150 ms after stimulus onset, which is considerably later than previously shown distractor suppressions in parietal cortex (Ipata et al., 2006) suggesting that top-down mechanisms might underlie the effects in visual cortex. Response times were furthermore negatively correlated with neuronal target responses, implying that neuronal target

facilitation improves target detection and response selection performance. The relatively little error trials in which animals selected the salient distractor instead of the target were characterized by weaker responses to target stimuli and markedly stronger responses to distractors. We did not observe any differences on behavioral or neuronal responses when we categorized trials according to preceding reward level and color changes, suggesting that the suppression and avoidance of salient distractors constitutes a higher priority for these highly trained animals than the amount of reward associated with a particular color. We conclude that the neuronal representation of bottom-up pop-out stimuli in visual cortex can be suppressed by top-down priority signals to facilitate goal-directed behavior.

Disclosures: P.C. Klink: None. **J.A.M. Lorteije:** None. **B. van Vugt:** None. **P.R. Roelfsema:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.01/R19

Topic: D.04. Vision

Support: NIH EY002966

ONR BAA08-019

Title: V2 neurons during free-viewing of static figure arrays: Surge of contour grouping at target of impending saccade

Authors: *L. A. ZHANG[1], R. VON DER HEYDT[2];
[2]Zanvyl Krieger Mind/Brain Inst., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Neurons in early visual cortex are selective for local visual features but their responses can also be influenced by border ownership and top-down selective attention. Most of what we know about the function of these neurons is based on neurophysiological studies in monkeys that hold their direction of gaze fixed while isolated visual stimuli are presented. However, during natural behavior, primates visually explore cluttered environments by changing gaze direction several times each second. We have previously proposed that border-ownership selectivity is best explained by a model in which contour selective neurons in low-level visual cortex are enhanced by feedback from grouping cells at a higher level. Object-based attention is thought to target these grouping cells. Thus, when a figure becomes the target of a saccade, this theory predicts that responses representing the figure contour should be enhanced, but only in neurons whose border ownership preferences point to the interior of the figure, because these are connected to the targeted grouping cell. We used a foraging task in which the monkey chooses where to look and when. Geometrical figures were displayed and the monkey was rewarded for

fixating the center of a figure for at least 200ms. In each trial an array of 10 figures (5 squares and 5 triangles) was presented, one of which was randomly associated with reward, and a cue informed the monkey whether it was a square or a triangle. The monkey sequentially fixated the cued shapes (and sometimes also the other shapes) in search for reward. Single neurons were recorded from area V2. After mapping the receptive field of a cell, the figure array was constructed so that fixating the center of a figure would, in most cases, bring an edge of another figure into the receptive field. We show that feature coding, border ownership selectivity and top-down modulation can be measured reliably with this method despite the fast pace of fixations (mean time between saccades about 350ms). Our results imply that contour grouping occurs in parallel for the objects in the display. Contour responses were further enhanced when the figure at the receptive field was the target of an impending saccade, but only in neurons with border-ownership preferences pointing to the interior of the figure, in support of the feedback grouping model. Thus, saccades are preceded by bursts of activity in neurons representing the contours of the selected object. We conclude that border ownership selectivity is the signature of grouping mechanisms that are fundamental for the individuation and perception of objects.

Disclosures: L.A. Zhang: None. **R. von der Heydt:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.02/R20

Topic: D.04. Vision

Support: NWO Brain and Cognition grant 433-09-208

ALW grant 823-02-010

ERC advanced grant Cortic_al_gorithms; no 339490

Title: Border ownership tuning predicts effective connectivity between V4 and V1 in macaque visual cortex

Authors: *M. W. SELF[1], D. JEURISSEN[1], A. F. VAN HAM[1], M. SENDEN[2], P. R. ROELFSEMA[1];
[1]NIN, Amsterdam, Netherlands; [2]Maastricht Univ., Maastricht, Netherlands

Abstract: The responses of cells in V1 are modulated by the perceived structure of the visual scene. Responses are higher on regions perceived as figures and lower on the background, an effect known as figure-ground modulation (FGM). This enhanced firing on figural surfaces is thought to group together different parts of the same object in perception. The mechanisms by which this modulation arises are unknown but they are thought to be due to feedback from higher

visual areas. Here we develop a new model of FGM in which connections between higher visual areas and V1 are governed by the border-ownership preferences of the cells in higher visual areas. Border-ownership (BO) is a property shown by many cells in V2 and V4 where the response of the cell to a boundary is modulated according to which side of the boundary is 'owned' by the figure. This property is thought to be important for grouping together the boundaries that belong to the same object. It is currently unknown how this modulatory effect relates to FGM. We propose that BO cells send feedback to V1 in the direction of their preferred side of figure while suppressing activity on the non-preferred side. We simulated a network of cells connected in such a way and demonstrate that it can reproduce the patterns of FGM observed in V1 for many types of figures. The model makes a key prediction that cells in higher visual areas will be more strongly connected to V1 cells with RFs located on their preferred side of ownership. We tested this prediction by making simultaneous multi-unit recordings from V1 and V4 of an awake behaving monkey. We observed many multi-units in V4 with strong BO preferences suggesting that cells with similar BO preferences may be spatially clustered in this area. We estimated connectivity using spontaneous noise correlations. We found that, as predicted by the model, noise correlations were strongest when the BO tuning pointed towards the spatial location of the V1 RF. Using the agreement between the BO tuning and the location of the V1 RF and the distance between the RF centers we could account for over 50% of the variance in spontaneous noise correlations between V4 and V1. Noise correlations were also modulated by figure-ground structure. Critically, noise correlations between V4 and V1 were highest when the V1 RF fell on a figure and the V4 RF was stimulated by its preferred boundary raising the possibility that the perceived structure in the scene enhances the effective connectivity between V4 and V1. The data suggest that FGM may arise through interactions between BO tuned cells in higher visual areas and cells in V1 leading to labeling of the surfaces of figures with enhanced firing-rates.

Disclosures: **M.W. Self:** None. **D. Jeurissen:** None. **A.F. van Ham:** None. **M. Senden:** None. **P.R. Roelfsema:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.03/S1

Topic: D.04. Vision

Support: NWO Vici

NWO MaGW

ERC

Title: The development of figure-ground segregation across time and space in the visual cortex of the macaque monkey

Authors: *D. JEURISSEN, A. F. VAN HAM, M. W. SELF, P. R. ROELFSEMA; Netherlands Inst. For Neurosci., Amsterdam, Netherlands

Abstract: The primate visual-system is well equipped to quickly recognize objects and segregate objects from their background. As visual information is sent to higher cortical areas, the complexity of the tuning of cells in the subsequent stages increases. Shape selectivity in ventral-stream areas is present very soon after stimulus onset. Some 50-60ms later, cells in primary visual cortex show an increased firing rate when their receptive field is on a figure compared to when it is on a background. This modulatory effect of contextual stimuli placed outside the classical receptive field is known as figure-ground modulation. Mid-level visual areas may play a crucial role in linking the information about the shape and identity of an object in ventral stream areas with the high-resolution information available in early visual areas. It has remained unclear how cells in mid-level visual-areas mediate interactions between lower and higher visual areas for figure-ground perception, what their degree of spatial selectivity is, and how this selectivity develops over time. To address these issues, we recorded multi-unit activity in mid-level visual area V4 in a macaque monkey while it viewed figure-ground stimuli. We mapped out the response to a square of different scales and defined by different cues. The initial responses showed a highly spatially specific response to boundaries. Surprisingly, the same multi-unit signals appeared to have large RFs when mapped with a small luminance defined square. At a later stage the response showed clear preferences for particular boundaries of the square, including cells that responded only to the corners. At even later times, the response surrounding the square became suppressed, creating a Mexican-hat profile. Overall we found little difference in the response profile between texture-defined and luminance defined shapes, suggesting that area V4 holds an abstract representation of the shapes that is relatively independent of low-level features. If the animal was required to make a saccade towards the figure this enhanced the representation of the interior of the figure. We conclude that V4 neurons encode different aspects of visual objects in different phases of their response in accordance with a crucial role in routing information between lower and higher visual areas.

Disclosures: D. Jeurissen: None. **A.F. van Ham:** None. **M.W. Self:** None. **P.R. Roelfsema:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.04/S2

Topic: D.04. Vision

Support: ERC advanced grant (Cortic_al_gorithms; no 339490)

Title: Global scene-interpretation affects figure-ground modulation and contrast perception

Authors: *A. F. VAN HAM, M. W. SELF, D. JEURISSEN, P. R. ROELFSEMA;
Netherlands Inst. For Neurosci., Amsterdam Zuidoost, Netherlands

Abstract: The process of figure-ground segregation requires local analysis of low-level features and global analysis of the visual scene in accordance with the Gestalt rules of visual perception. However, the influence of global scene-interpretation has been debated. Neural correlates of figure-ground segregation have been found in visual areas as early as V1. Neurons in macaque V1 show enhanced responses to figures compared to backgrounds, an effect known as figure-ground modulation (FGM). This suggests that global scene analysis influences activity at the earliest cortical processing stages. However, FGM has typically been measured using relatively small figures and therefore the possibility remains that FGM arises through local computations. Recent studies suggested that the impact of FGM is profound, because it may even influence the perception of contrast. However, also for this effect the possibility remains that it arises through local computations within early visual cortex. To address this issue, we studied whether global Gestalt laws influence perceived contrast in humans and monkeys and whether it causes FGM by recording multi-unit activity in V1 and V4 of monkeys. On each trial, we presented two horizontal strips of texture-defined shapes. One strip without Gestalt cues served as the baseline. In the other strip the Gestalt rules enclosure, convexity, symmetry, or the combination of these three cues induced a figure-ground organization. A contrast discrimination task performed on two superimposed Gabors allowed us to investigate perceived contrast. The behavioral results showed that the humans and monkeys perceived Gabors on figures as higher in contrast than Gabors on the background, suggesting that global scene-interpretation influences contrast perception. In humans, the change in perceived contrast depended on their subjective report of "figureness". These behavioral results were well explained by the neural data in V1 and V4 of the monkeys. We observed enhanced neural activity in V1 and V4 when receptive fields fell on figures compared to backgrounds. FGM occurred earlier in V4 than in V1, suggesting that global scene information from higher visual areas is fed back to lower visual areas to establish FGM. In addition, the Gestalt cues induced FGM of different strengths with strongest FGM when cues were combined, and the strength of FGM predicted the change in perceived contrast. We conclude that global scene-interpretation influences both FGM and contrast perception. These results imply that FGM is a global phenomenon that requires computations beyond the scope of early visual areas and presumably rely on feedback from higher visual areas.

Disclosures: A.F. Van Ham: None. **M.W. Self:** None. **D. Jeurissen:** None. **P.R. Roelfsema:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.05/S3

Topic: D.04. Vision

Support: Grant-in-Aid for Scientific Research on Innovative Areas, Sparse Modeling (25120004), from the Japan Society for the Promotion of Science (JSPS)

Grant-in-AID for Scientific Research (26240021)

RIKEN Junior Research Associate Program

Title: Distinct computational modules for forming central and peripheral parts of a receptive field in monkey anterior inferior temporal cortex

Authors: K. OBARA[1,2], *K. O'HASHI[1], M. TANIFUJI[1,2,3];
[1]Brain Sci. Institute, RIKEN, Saitama, Japan; [2]Dept. Life Sci. Med. Biosci., Waseda Univ., Tokyo, Japan; [3]Dept. Complexity Sci. and Eng., Grad. Sch. of Frontier Sciences, Univ. of Tokyo, Tokyo, Japan

Abstract: A remarkable aspect of object recognition is that we recognize a few objects embedded in a cluttered environment in our daily life.To recognize these objects unambiguously, it would be advantageous to have receptive fields (RFs) as small as the target objects. On the other hand, explanation of translational invariance in object recognition requires RFs to be large. In inferior temporal (IT) cortex of macaque monkeys, previous studies have shown that IT neurons have large RFs (ex, Kobatake and Tanaka, 1994; Op de Beeck and Vogels, 2000). For example, one of the studies with monkeys performing a fixation task showed the mean size of RFs to be as large as 10.3 degree (Op de Beeck and Vogels, 2000). On the other hand, another study reported that RF can be as small as 2.6 degree when monkeys performed a task to recognize a small stimulus presented near the fixation point (DiCarlo and Maunsell, 2003). Based on these findings, we believe that IT neurons have large RFs in a default mode, such as the RFs observed in monkeys with the above fixation task, and that the default mode RFs can be modulated by task demands. Modulation of the default mode RFs may be critical for explaining object recognition in a cluttered environment. Along this line, we have investigated neural circuit mechanisms along the ventral visual pathway for formation of the default mode RFs and modulation of the RFs with spatial attention as a factor associated with task demands. We have already reported that (1) central part of a RF of anterior IT neurons was processed significantly faster than peripheral part, (2) this difference in processing latency did not depend on spatial attention, but (3) the magnitudes of responses at different locations in RFs were modulated depending on the location of spatial attention (Obara, O'Hashi, and Tanifuji, Cosyne Abstracts 2015, Salt Lake City USA.). In the present study, to test whether these properties exist before visual information reaching IT cortex, we examined latency and attentional modulation of V4 neurons with RFs in peripheral and central parts of the visual field respectively. As a result, we found that differences in latency and attentional modulation of magnitude were not observed in V4 neurons. Thus, default mode processing of objects in central visual field and of objects in

peripheral visual field use different computational modules in areas between V4 and anterior IT (potentially, area TEO), and the attention modulates the output gain of the modules.

Disclosures: **K. Obara:** None. **K. O'Hashi:** None. **M. Tanifuji:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.06/S4

Topic: D.04. Vision

Title: Temporal dynamics of visual category representation in the macaque inferior temporal cortex

Authors: *M.-R. ABOLGHASEMI-DEHAQANI[1], A.-H. VAHABIE[1], R. KIANI[1,2], M. NILI AHMADABADI[1,3], B. NADJAR ARAABI[1,3], H. ESTEKY[1,4];
[1]IPM, Tehran, Iran, Islamic Republic of; [2]NewYork Univ., NewYork, NY; [3]Univ. of Tehran, Tehran, Iran, Islamic Republic of; [4]Shaheed Beheshti Univ. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract: Object categories are recognized at multiple levels of hierarchical abstractions. Psychophysical studies have shown a more rapid perceptual access to the mid-level category information (e.g. human faces) than the higher (superordinate; e.g. animal) or the lower (subordinate; e.g. face identity) levels. Mid-level category members share many features while few features are shared between members of different mid-level categories. To better understand the neural basis of expedited access to mid-level category information we examined neural responses of the inferior temporal (IT) cortex of macaque monkeys viewing a large number of object images. We found an earlier representation of mid level categories in the IT population and single unit responses compared to superordinate and subordinate level categories. The short latency representation of mid-level category information shows that visual cortex first divides the category shape space at its sharpest boundaries defined by high/low within/between group similarity. This short latency mid-level category boundary map may be prerequisite for representation of other categories at more global and finer scales.

Disclosures: **M. Abolghasemi-Dehaqani:** None. **A. Vahabie:** None. **R. Kiani:** None. **M. Nili Ahmadabadi:** None. **B. Nadjar Araabi:** None. **H. Esteky:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.07/S5

Topic: D.04. Vision

Support: NSFC grant 61473169

CPSF grant 2012M510398

Title: Recurrent processing of object category and identity in human visual cortex

Authors: *R. XU[1,2], X. YU[3], H. ZUO[4], H. LIU[5], B. HONG[1];
[1]Dept. of Biomed. Engineering, Sch. of Med., Tsinghua Univ., Beijing, China; [2]McGovern Inst. for Brain Res. at MIT, Cambridge, MA; [3]Dept. of Neurosurg., Chinese PLA Gen. Hosp., Beijing, China; [4]Dept. of Neurosurg., Yuquan Hospital, Tsinghua Univ., Beijing, China; [5]Athinoula A. Martinos Ctr. for Biomed. Imaging, Dept. of Radiology, Massachusetts Gen. Hosp. & Harvard Med. Sch., Charlestown, MA

Abstract: The ventral visual areas play a key role in object recognition and may act as the "object center" in the human brain. However, how the higher-order object information (e.g., category and identity) is extracted in these areas remains elusive: is this accomplished via pooling feedforward input from lower visual areas, or through inter- and intra-areal recurrent interactions? To address this question, here we recorded electrocorticography (ECoG) activity from fourteen epilepsy patients and investigated the spatiotemporal dynamics of object processing in both early visual cortex (EVC) and occipito-temporal cortex (OTC) along the ventral pathway. Each patient was presented 120 images of faces, places, and common objects (categories), which in turn can be subcategorized to male/female faces, houses/scenes, cars /chairs, respectively (classes). Class-specific responses were observed in both areas (EVC, n=53 electrodes; OTC, n=61) and object classes could be decoded on a single-trial basis. Consistent with the anatomically defined hierarchy, class recognition was earlier in EVC than OTC. We used representational dissimilarity matrices (RDM, at the level of classes) to characterize the time-varying neural codes in both areas. About 60 ms after stimulus onset, EVC built an RDM very similar to an image-based RDM (derived from pixel difference) whereas OTC showed a three-category RDM around 130 ms, consistent with previous observations. However, we found a categorical RDM emerged in EVC ~50 ms following the OTC processing, which was also invariant to non-categorical transforms (subcategory and image size). Additionally, the RDM in OTC was substantially refined over time both in structure (along the same trend as EVC with increasing processing order) and reliability (which drastically improved at the single trial level). Finally, we showed that OTC processed object category and identity at separate time windows: the identity-related activity peaked ~200 ms later than the category-related activity. In contrast, EVC processed various levels of information almost at the same time, with a much smaller yet opposite difference in latency (i.e. identity before category). Taken together, our data suggest a possible recurrent mechanism for cortical object processing: OTC first sums over EVC inputs to form a crude version of object code; and then the initial code is refined to enable reliable categorical representation, likely contributed by recurrent involvement of EVC. The more

specific information (i.e., object identity) is fully analyzed later, probably through interactions among and within high-level object areas.

Disclosures: **R. Xu:** None. **X. Yu:** None. **H. Zuo:** None. **H. Liu:** None. **B. Hong:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.08/S6

Topic: D.04. Vision

Support: KAKENHI(25350997)

KAKENHI(25135716)

Title: Information flow dynamics in inferior temporal cortex involving visual object processing

Authors: ***K. KAWASAKI**[1], T. HARUNA[2], H. SAWAHATA[3], H. TANIGAWA[1], A. IIJIMA[1], T. SUZUKI[4], I. HASEGAWA[1];
[1]Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata, Japan; [2]Dept. of Planetology, Kobe Univ., Kobe, Japan; [3]Toyohashi Univ. of Technol., Toyohashi, Japan; [4]Natl. Inst. of Information and Communications Technol., Osaka, Japan

Abstract: Converging evidence suggests that cortical visual processing is performed in a hierarchical network. In the primate object vision, visual information is thought to be processed along the following sequence of areas: V1, V2, V4, posterior part of the inferior temporal cortex (pIT), central part of IT (cIT) and anterior part of IT (aIT). Nonetheless, the direct physiological insight on the hierarchical processing has been constrained due to the lack of appropriate techniques. In particular, it is difficult to record neural activity simultaneously from wide cortical areas at high temporal resolution. Here we conducted electrocorticography using a 128-channel 2.5 mm spaced surface electrode, which covered ventral visual areas of pIT, cIT and aIT while the monkeys performed a passive visual fixation task to investigate how the process was embedded in spatiotemporal activities of these areas. The high spatiotemporal resolution recording captured dynamic propagation of the visually evoked activity and identified three repeated waves traveling from pIT to aIT after the stimulation onset. To quantify these activity dynamics, we employed symbolic local transfer entropy (SLTE) extracting spatiotemporal pattern in terms of information measure. SLTE quantified information flow between all neighbor electrodes then information potential was defined from the flow. We identified feedforward and feedback components, the information flow directing pIT to aIT and the reversed flow, respectively. The first feedback flow appeared as early as 60ms after onset of the visual stimulation. The feedforward flow appeared and peaked around 100-130ms after the onset followed by second feedback flow. Stimulus selectivity emerged during these fast information

traffic suggesting the importance of both feedforward and feedback information to generate visual selectivity. Furthermore by examining dynamics of the SLTE topography, we found this hierarchical network changed its shape drastically during the visual stimulation. A few specific spots oscillated as the information sink and source more significantly than the others. Small-world-ness measures indicated the network decreased its local connectivity and increased its global connectivity during visual stimulation, suggesting an enhancement of the global information integration. The spatial shuffling procedure conformed the necessity of the organized two-dimensional activity to acquire these network properties. These results provide the physiological basis of the dynamic hierarchical processing in the ventral visual stream and suggest that this process involves topographic changes in the information network.

Disclosures: **K. Kawasaki:** None. **T. Haruna:** None. **H. Sawahata:** None. **H. Tanigawa:** None. **A. Iijima:** None. **T. Suzuki:** None. **I. Hasegawa:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.09/S7

Topic: D.04. Vision

Support: ERC Grant 249640

Title: Hierarchical feed-forward visual models and recurrent semantic models predict fMRI pattern-information in the ventral object processing stream

Authors: ***B. J. DEVEREUX**[1], A. CLARKE[2], L. K. TYLER[1];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Understanding visual objects involves both hierarchically-organized feed-forward processing and later more recurrent processing that yields detailed and perceptually-invariant information about object meaning (Schendan & Stern 2008; Clarke et al 2014). Convolutional neural networks have provided plausible models of the first part of this system, with successive layers of such networks capturing increasingly high-level visual information about the input. However, how such processing activates and interacts with semantic representations remains unclear. We combined a deep convolutional network model of vision (Krizhevsky et al 2012) with an attractor network model of concept semantics (Cree et al 2006) where information about object meaning is represented as a pattern of activation across distributed feature units. This integrated visuo-semantic model maps high-level visual representations onto semantic feature representations, encoding statistical information about semantic features (e.g. feature frequency) and their relationship to high-level visual information. We found that early stages of the semantic

model showed stronger activation for features shared by many objects and features which are visual in nature (e.g. “is long”), compared with distinctive and non-visual features. Using RSA (Kriegeskorte et al 2008), we tested the ability of the model to explain patterns of activation in fMRI data where 16 participants named pictures of 131 objects (Clarke & Tyler 2014). We calculated 8 dissimilarity matrices (DMs) corresponding to the 8 layers of the deep convolutional network and 20 DMs corresponding to the 20 processing stages of the attractor network. The 28 DMs delineate a trajectory through a space of representational geometries, from pixels to detailed object semantics. Layers of the deep convolutional network explained pattern similarities in early visual cortex, consistent with previous results (Khaligh-Razavi & Kriegeskorte 2014). However, DMs corresponding to the early stages of the attractor network, where activation of shared and visual semantic features is strong relative to non-visual and distinctive features, better explained pattern similarity in the posterior fusiform. The final stage of the semantic attractor network model, where detailed semantic representations, including both shared and distinctive features, are maximally activated, best explained pattern similarity in bilateral perirhinal cortex. Taken together, the results show how models integrating visual and distributed semantic representations can account for fMRI pattern-information throughout the ventral temporal object processing stream.

Disclosures: **B.J. Devereux:** None. **A. Clarke:** None. **L.K. Tyler:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.10/S8

Topic: D.04. Vision

Support: R01 EY021717

R01 NS065049

Title: The role of action information in thematic relations between objects

Authors: R. E. WITTENBERG[1], C. E. WATSON[2], L. J. BUXBAUM[2], *S. L. THOMPSON-SCHILL[1];
[1]Univ. Pennsylvania, Philadelphia, PA; [2]Moss Rehabil. Res. Inst., Philadelphia, PA

Abstract: Recent evidence suggests that object concepts may be organized both taxonomically (categorically) and thematically (in terms of associated roles in events). Thematic information, in particular, appears to be crucial for determining relationships between manipulable objects. This suggests that action information may play a role in the processing of thematic relationships between objects. One previous study found that stroke patients with lesions to left temporo-parietal cortex were less sensitive to the “action-relatedness” of thematically-related manipulable

objects than healthy controls or patients with other lesion loci, consistent with a critical role of this region in action-based object relationships. In the present study, we used fMRI with healthy participant (n = 13) to further investigate the role of action in semantic relations and their neural bases. On each trial, participants saw a triad of object photographs: a target object (top) and two related objects (bottom left and right). One of the related objects was always related to the target taxonomically ("Tax", wine/water bottle). The other related object was related thematically via a common action ("Th+A", e.g., wine/corkscrew) or thematically via co-occurrence in space and time rather than a common action ("Th-A", e.g., wine/cheese). Participants performed two tasks on these triads. During the Category task, participants selected the object that was taxonomically related to the target. During the Event task, participants selected the object that participates in the same event or scene as the target. We also included a baseline task in which participants performed an identity match with scrambled versions of the object triads. Comparison of all trials minus baseline showed greater activation in bilateral inferior parietal lobe and lateral occipitotemporal cortex. During the Event task, activation was greater in bilateral lateral occipitotemporal and medial fusiform cortex when judgments involved objects related via a common action versus objects related via co-occurrence in space and time (Th+A vs. Th-A). These data are consistent with an important role of these regions in action-based thematic object relationships.

Disclosures: R.E. Wittenberg: None. **C.E. Watson:** None. **L.J. Buxbaum:** None. **S.L. Thompson-Schill:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.11/S9

Topic: D.04. Vision

Support: NSERC Discovery + Accelerator Grant (249877-2006-RGPIN)

Title: fMRI reveals representational similarity for objects that are used on the body vs. other objects

Authors: *S. D. SQUIRES[1], J. C. SNOW[2], K. M. STUBBS[1], J. C. CULHAM[1];
[1]The Brain and Mind Inst., Univ. of Western Ontario, London, ON, Canada; [2]Psychology, Univ. of Nevada, Reno, NV

Abstract: Neuroimaging research has revealed numerous brain regions that are more activated by viewing tools vs. other categories of visual stimuli (e.g., animals or non-tool objects). Here we investigated the neural representations for tools and other objects presented as either real objects or photos of those same objects. We wondered whether tool-selectivity would be

enhanced for real tools vs. photos of tools (to a greater degree than real non-tools vs. photos of non-tools) because real stimuli afford actual actions. Fifteen participants viewed real objects or photos matched for size and viewpoint. Individual objects and photos were mounted on semicircular plates (designed to abut the top of the scanner bore) and successively presented using a custom-designed conveyor belt called the DROID (Delivery of Real Objects for Imaging Device). Items were either tools (plastic whisks, toothbrushes or hammers) or non-tools of comparable size and elongation (socks, sunglasses and candles). Within each block, subjects viewed four exemplars (of different color, form, etc.) of one object type (e.g., whisks) in either real or photo format. Much to our surprise, we did not find higher activation for tools than non-tools (regardless of format). Interestingly however, data-driven multivariate representational dissimilarity analyses revealed an unexpected but interesting grouping of categories. Specifically, multidimensional scaling of the neural representations showed that the representations of socks, sunglasses, and toothbrushes were more similar to each other than to another grouping of hammers, whisks, and candles. These data suggest that tool-selective activation may be quite dependent on the choice of control category and driven in part by low-level factors like object size, elongation, and or actability. More interestingly, they reveal a new theoretical dimension that may account for differences in representations in the ventral visual stream: objects that are used to interact with the body may invoke different representations than objects that interact with other objects.

Disclosures: **S.D. Squires:** None. **J.C. Snow:** None. **K.M. Stubbs:** None. **J.C. Culham:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.12/S10

Topic: D.04. Vision

Title: Localizing tool- and hand-selective areas with fMRI: Comparing video and picture stimuli

Authors: *S. N. MACDONALD[1], F. M. Z. HEILIGENBERG[2], T. R. MAKIN[2], J. C. CULHAM[1];
[1]Neurosci., Western Univ., London, ON, Canada; [2]Nuffield Dept. of Clin. Neurosciences,, Univ. of Oxford, FMRIB, Oxford, United Kingdom

Abstract: Historically, tool- and hand-specific areas have been localized in the human brain by using functional magnetic resonance imaging (fMRI) to compare brain activity when participants view pictures of tools or hands versus when they view pictures of objects or scrambled images. In contrast to pictures, however, videos can fully depict the interaction between an effector and its target and likely engage attention to a greater extent. Thus, we hypothesized that videos would be more effective than pictures at localizing distinct tool- and hand-selective areas within

individuals, and videos would also be more likely to recruit areas involved in tool and hand actions. Ten healthy participants underwent fMRI while they viewed pictures and videos presented in a blocked design. Picture stimuli included tools, hands, objects, and scrambled images. Likewise, video stimuli included tools interacting with objects (hand omitted from the scene), hands interacting with objects, moving objects, and moving patterns (akin to scrambled images). The results show that tool and hand videos activate a more extensive network of dorsal stream areas compared to pictures. Specifically, areas in the action observation and execution network were more selective for tool and hand videos as compared to object videos. The robust activation elicited by videos facilitated the localization of tool- and hand-selective areas at the individual level. Interestingly, tool- and hand-selective areas localized using videos included the anterior portion of the lateral occipital temporal cortex (LOTC), which is selective for actions. In contrast, pictures widely activated a posterior part of LOTC associated with motion processing. In sum, videos are effective stimuli for localizing areas involved in the processing of tools and hands. Videos reliably activate tool- and hand-selective regions and, compared to pictures, better recruit areas likely involved goal-directed action.

Disclosures: **S.N. Macdonald:** None. **F.M.Z. Heiligenberg:** None. **T.R. Makin:** None. **J.C. Culham:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.13/S11

Topic: D.04. Vision

Support: JSPS KAKENHI Grant 15H05311

JSPS KAKENHI Grant 26120536

JSPS KAKENHI Grant 15K16017

NIH Grant EY019684

NSF Grant CCF-0939370

William Orr Dingwall Neurolinguistics Fellowship

Title: Word statistics in lager-scale texts explain the human cortical semantic representation of objects, actions, and impressions

Authors: ***S. NISHIDA**[1,2], A. G. HUTH[3], J. L. GALLANT[3], S. NISHIMOTO[1,2];
[1]Ctr. for Information and Neural Networks, Natl. Inst. of Info. and Comm. Technol., Suita, Osaka, Japan; [2]Osaka Univ., Suita, Osaka, Japan; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Words in natural language have semantic structures that reflect the relationship between thousands of different semantic categories such as objects, actions, and impressions. Recently many language models have been proposed in order to extract such semantic structures from large-scale text corpora in an unsupervised fashion. Of these, the skip-gram learning algorithm has attracted broad interest because it constructs a semantic vector space that allows explicit linear translations of word representation in a manner congruent with our intuition (e.g., King - Man + Woman = Queen). To investigate whether and how the skip-gram vector space is reconciled with the semantic representation in the human brain, we built a voxelwise encoding model of human brain activity based on the skip-gram vector representation (skip-gram model). In order to test the validity of the model, we recorded whole-brain BOLD (blood oxygen level-dependent) activity of human subjects who watched natural movies and fit the model to individual voxels. We found that the skip-gram model predicted movie-evoked activity in many cortical areas, particularly in the occipitotemporal visual areas. The skip-gram model outperformed other corpus-based language models in terms of prediction accuracy. Analysis of the fit models revealed structured cortical semantic spaces associated with the meanings of words separately for objects (nouns), actions (verbs), and impressions (adjectives). Moreover, by using the skip-gram vector representation, we could also decode semantic contents of perceived movie scenes from the evoked activity. These results suggest that the skip-gram vector representation captures important features of the semantic representation in the human brain.

Disclosures: **S. Nishida:** None. **A.G. Huth:** None. **J.L. Gallant:** None. **S. Nishimoto:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.14/S12

Topic: D.04. Vision

Title: Object-to-spatial property "crosstalk" improves scene recognition: a modeling study

Authors: *D. LINSLEY, S. MACEVOY;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Scene recognition is a key function of the visual system, supporting effective navigation through one’s surroundings and appropriate interactions along the way. Scene recognition mechanisms operate both on the objects within a scene and the scene’s spatial properties, with a single judgment of scene identity ultimately emerging from a combination of the two. We recently demonstrated that this convergence of object and spatial information begins in the visual system, with scenes’ encoded spatial scales biased towards the scales associated with the objects they contain (Linsley & MacEvoy, 2014). For instance, the spatial scale of a room containing an oven is perceived as more “kitchen-like” than if the oven were not present.

We have theorized that this bias aids scene recognition by harmonizing scenes' encoded spatial properties with their perceived object contents. In the present study, we used artificial neural networks to test the plausibility of this theory. Separate models simulated scene recognition based solely on scenes' objects, spatial properties, or a pooled combination of these sources that permitted but did not mandate a bias consistent with our previous observations. Consistent with representations of scenes inferred from behavior and measured with fMRI in the parahippocampal place area, the model that pooled object and spatial property information encoded scenes as more spatially similar to their category average when objects were visible than when they were obscured by wavelet masks. This model also produced similar errors as humans on a scene categorization pilot study, both when scene exemplars were intact and when their objects were masked. Finally, we observed that the model permitting bias yielded better scene recognition accuracy than the other two models. Additionally, because the model permitting bias pools information across objects spatial properties, redundant features were pruned, resulting in significantly more efficient scene representations. In summary, our model supports the theory that the perceptual convergence of information about scenes' objects and spatial properties benefits scene recognition by improving both accuracy and efficiency.

Disclosures: D. Linsley: None. **S. MacEvoy:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.15/S13

Topic: D.04. Vision

Support: NIMH Intramural Research Program

Title: Differential representation of man-made and natural scenes in scene-selective cortex

Authors: *I. I. GROEN, E. H. SILSON, C. I. BAKER;
Natl. Inst. for Hlth., Bethesda, MD

Abstract: Behavioral, computational and electroencephalography (EEG) results suggest that man-made and natural scenes are represented differentially (Oliva and Torralba, 2003; Greene et al., 2009; Groen et al., 2013), but the neural substrate of this global scene distinction is unclear. Three scene-responsive brain regions - the parahippocampal place area (PPA), transverse occipital sulcus (TOS) and retrosplenial complex (RSC) - have shown to be sensitive to various visual features that may contribute to scene naturalness, such as spatial layout, landmarks, object ensembles, and texture (Kravitz et al., 2011, Park et al., 2011, Epstein et al., 2014, Cant and Xu, 2011) as well as low-level properties such as spatial frequency and contrast (Rajimehr et al., 2011, Kaufmann et al., 2015). Here, we used high-resolution (7T) fMRI to examine neural

representations of man-made versus natural scenes and to characterize how these representations relate to potentially diagnostic scene features. As an initial test, we used a man-made/natural scene localizer across the whole brain, which revealed patches of enhanced activity for man-made compared to natural scenes within each of the three scene-responsive areas. Interestingly, in PPA, this patch was located anteriorly and medially, suggesting that in 3T data, it may have been obscured by increased susceptibility artifacts. Given this difference in univariate responses, we sought to investigate what scene information contributes to this difference using multi-voxel pattern analysis across 4 scene (man-made open, man-made closed, natural open, natural closed) and 4 isolated object (man-made, natural, buildings and faces) categories. The results revealed heterogeneous response profiles across the scene-responsive regions. Anterior PPA and RSC not only separated man-made from natural scenes but also man-made from natural objects, and man-made scenes clustered with buildings more strongly in anterior compared to posterior PPA. In contrast, TOS did not distinguish between isolated object categories, but did have a selectively distinct representation of closed natural scenes (which contain more textural components) compared with the other scene types. Preliminary results of consecutive transcranial magnetic stimulation (TMS)/fMRI indicate that TMS to TOS eliminates its man-made advantage, while the response in PPA remains largely intact. Together, these findings suggest that man-made and natural scenes could be differentially represented based on both object-based and textural properties, which are potentially extracted via distinct pathways involving PPA and TOS, respectively.

Disclosures: **I.I. Groen:** None. **E.H. Silson:** None. **C.I. Baker:** None.

Poster

333. Visual Processing: Object and Scene Representation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 333.16/S14

Topic: D.04. Vision

Support: SFB 874

Title: In search of categorical object representation in the pigeon's nidopallium frontolaterale

Authors: ***C. KOENEN**, R. PUSCH, F. BROKER, S. THIELE, O. GUNTURKUN; Ruhr-University Bochum, Bochum, Germany

Abstract: How do pigeons see the world? Are visual objects represented in a similar manner in the avian visual system compared to the primate visual system? To elucidate these questions we examine the pigeon's nidopallium frontolaterale (NFL), a higher visual area and the putative analog to the inferior temporal (IT) cortex of primates. To investigate the visual response properties of the NFL we present different stimuli sets to freely moving and behaving pigeons

while recording single cell activity from the NFL. We present basic visual stimuli, namely simple forms in different colors as well as gratings with varying spatial frequencies and contrasts. Additionally, we show pictures of real-world objects from different categories. Kriegeskorte et al. (2008) presented the same pictures of real-world objects to humans and monkeys and revealed an activity pattern in IT in line with semantic categories. Moreover, the category clusters where highly similar between man and monkey, recorded with fMRI and single cell recording, respectively. With our approach we investigate the neural basis of perceptual discrimination in the NFL on different levels of complexity and compare the results to object representation in primate IT. References Kriegeskorte, N., Mur, M., Ruff, D. A., Kiani, R., Bodurka, J., Esteky, H., . . . Bandettini, P. A. (2008). Matching categorical object representations in inferior temporal cortex of man and monkey. Neuron, 60(6), 1126-1141. doi: 10.1016/j.neuron.2008.10.043

Disclosures: C. Koenen: None. **R. Pusch:** None. **F. Broker:** None. **S. Thiele:** None. **O. Gunturkun:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.01/S15

Topic: D.06. Eye Movements

Support: CAS Hundred Talent Program

Title: Role of superior colliculus in microsaccade generation as examined with electrical microstimulation

Authors: M. C. DORRIS, P. BAO, M. YANG, *G. YU;
Inst. of Neuroscience, Shanghai, Shanghai, China

Abstract: During attempted fixation, the eyes continue to make tiny saccades (typically <1°), called “microsaccades”. Previous studies found that neurons in the rostral pole of the primate superior colliculus (SC) display a burst of activity associated with microsaccade generation and pharmacological inactivation of SC can bias microsaccade direction away from the affected region. Here, we systematically manipulated activity with varying levels of electrical microstimulation at a range of locations across the SC map. Monkeys simply fixated a central stimulus for 2500ms. We applied microstimulation (0.25ms biphasic pulses) to the SC intermediate/deep layers for 500ms during the middle of this fixation period. The stimulation intensity for each experiment was determined by the current level (at 300Hz) required to consistently evoke saccades to the stimulation site. Stimulation frequency was then randomly varied across 7 levels: 0Hz (control), 10Hz, 25Hz, 50Hz, 75Hz, 150Hz and 300Hz. The

stimulation sites spanned the SC ranging from 0.2° - 30° saccade eccentricities. There were three main findings: 1) there was a transition from fixational microsaccades at low levels of stimulation to macrosaccades at the highest levels of stimulation, 2) low level stimulation biased microsaccade direction towards the stimulation site without otherwise changing microsaccade frequency, amplitude or velocity. The rostrocaudal location of the stimulation site had no effect, 3) the above two findings were further modulated by the recency of naturally-occurring microsaccades relative to the time of stimulation onset. This provides support for the hypothesis of an underlying rhythmicity within SC circuitry that influences microsaccade timing. In conclusion, our results demonstrate that direct low level SC activation biases the direction of microsaccades without affecting other motor properties of these movements. This suggests that previously documented sensory (e.g. onset of visual stimuli) and cognitive (e.g. visuospatial attention) effects on microsaccades generation may be mediated by their spatially specific activations upon the SC map.

Disclosures: **M.C. Dorris:** None. **P. Bao:** None. **M. Yang:** None. **G. Yu:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.02/S16

Topic: D.06. Eye Movements

Support: NIH Grant EY023277

Title: Visual error signal in the superior colliculus reflects the speed of saccade adaptation

Authors: *Y. KOJIMA;
Physiol. & Biophysics, Univ. of Washington, Seattle, WA

Abstract: Error signal that instructs cerebellar motor learning is calculated from the mismatch between the desired movement and the actual movement. In the Marr and Albus model, the error signal modulates climbing fiber activity that changes the synaptic strength of the parallel fibers on Purkinje cells. This model is known as cerebellar plasticity. Various neurophysiological studies of saccade adaptation support this model. The climbing fibers that project to the cerebellar oculomotor vermis (OMV) elicit Purkinje cell complex spikes that are correlated with visual error during saccade adaptation. Simple spike activity of OMV Purkinje cells changes during the adaptation. The superior colliculus (SC) via the medial accessory olive is the putative signal source of the OMV climbing fibers. Subthreshold electrical stimulation of the SC timed after the end of each saccade produces saccade adaptation. The same electrical stimulation also elicits Purkinje cell complex spikes in the OMV. Taken together, this evidence suggests that the SC is the origin of the OMV complex spikes that drives saccade adaptation. In this study, we

recorded the SC activity related to the visual error signal during saccade adaptation. We induced adaptation of 15° saccades along a neuron's optimal direction. The intrasaccadic step (ISS) paradigm produced a fixed visual error that was congruent with the neuron's optimal vector. Throughout the adaptation, although the visual error was fixed, the visual activity of the SC modulated proportionally to the natural variation of the speed of the adaptation. When the saccade amplitude adapted faster, the visual activity increased, and vice versa. Although the visual activity of the neuron highly correlated with the speed of adaptation, the corrective-saccade-related bursts did not. Because the timing of Purkinje-cell complex spikes in the OMV coincides with the SC visual activity (~80ms after the offset of the primary saccades), the visual signal from the SC may modulate the activity of complex spikes, therefore, it may also modulate the speed of adaptation.

Disclosures: Y. Kojima: None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.03/S17

Topic: D.06. Eye Movements

Support: NIH Grant EY014885

Title: Local field potential signals related to saccade target selection in the superior colliculus

Authors: B. COOPER[1], *R. M. MCPEEK[2];
[1]Grad. Ctr. for Vision Res., [2]Biol. Sci., SUNY Optometry, New York, NY

Abstract: Neural activity related to saccade target selection in the primate superior colliculus (SC) has been studied extensively using single unit recordings, but few studies have investigated the local field potential (LFP) correlates of target selection in the SC. To investigate this, we trained monkeys in a top-down cueing task, in which physically identical potential saccade targets were initially presented in the periphery. After a delay, a cue at the fovea indicated which peripheral stimulus was the correct saccade goal. After another variable delay, the fixation point was extinguished and monkeys were rewarded for making a saccade to the cued stimulus. The delay periods allowed us to separate selection-related activity from movement-related activity. In the time domain, many, but not all, recording sites showed significant LFP modulation related to the onset of the foveal cue which indicated the correct target. These sites showed an increase in negativity when the cued target was in the response field (RF) vs. outside the RF. Other sites showed little response to the cue, but a significant increase in negativity occurring near the time of the saccade when the saccade goal was in the RF vs. out of the RF. In the frequency domain, in association with target selection in this task we saw an increase in power in the gamma band at

approximately 40-50 Hz, with little change in power in the beta band. This pattern of results contrasts with our findings in a pop-out color oddity selection task, reported last year, where saccade target selection was accompanied by an increase in power in the beta band (at approximately 25 Hz) in addition to an increase in gamma. To determine whether this difference was due to sampling of different LFP sites for the two tasks, we recorded LFP activity in both tasks at a small number of sites, and found similar frequency domain differences related to target selection in the two tasks. This suggests that signals related to bottom-up and top-down selection are processed differently in the SC, and that analyses of LFPs may help to distinguish these different components.

Disclosures: B. Cooper: None. **R.M. McPeek:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.04/S18

Topic: D.06. Eye Movements

Support: NIH R01 EY022854

NIH R01 EY024831

ERC Grant POSITION (P. Cavanagh)

Title: The motor burst of saccade-related neurons in the deep superior colliculus during interceptive saccades

Authors: *L. GOFFART[1], A. L. CECALA[2], N. J. GANDHI[3];
[1]CNRS, Marseille, France; [2]Elizabethtown Col., Elizabethtown, PA; [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Neurons in the deep layers of the superior colliculus (dSC) emit a vigorous burst of action potentials during saccades toward a stationary target. A traditionally defined movement field identifies an optimal saccadic vector for which the neural discharge is maximal, and the level of activity decreases for saccades gradually deviated from this optimum. Neurons in the dSC also produce a burst during interceptive saccades made to foveally acquire a moving target. Keller et al. (1996) described peri-saccadic bursts during interceptive saccades aimed at targets that moved away from the initial fixation point and through the neuron's response field at a constant velocity (usually 60 deg/sec). Two salient observations were reported: 1) the dSC's movement field center was shifted towards larger amplitude saccades relative to the center of movement fields collected using stationary targets; 2) the peak discharge for interceptive saccades tended to be lower than for saccades to stationary targets. To further characterize the

apparent discrepancy in the bursts for saccades to stationary and moving targets, we recorded activity of dSC neurons in three head-restrained rhesus monkeys trained to produce interceptive saccades toward targets traveling in numerous paths and at various speeds. In all cases, the target travelled through the neuron's traditionally-defined movement field. Consistent with Keller and colleagues, we observed less vigorous peri-saccadic bursts during interceptive saccades, and this attenuation is associated with a reduction in peak velocity. However, we did not observe a robust shift in the movement field's optimal vector or boundaries for the slower target speeds used in our experiments. Furthermore, if a shift did occur it was not always symmetrical for targets moving towards or away from the center of the traditional movement field. These data demonstrate that dSC is involved in the generation of interceptive saccades to moving targets. Further analyses are required to confidently decipher whether and how much target motion-related signals contribute to the burst.

Disclosures: **L. Goffart:** None. **A.L. Cecala:** None. **N.J. Gandhi:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.05/S19

Topic: D.06. Eye Movements

Support: Grant-in-Aid for Scientific Research on Innovative Areas ("Adaptive Circuit Shift")

Title: Selective optogenetic activation of output pathways from mouse superior colliculus

Authors: ***K. ISA**[1], T. SOOKSAWATE[2], K. KOBAYASHI[3], T. ISA[1,4];
[1]Natl. Inst. Phisiol. Sci., Okazaki, Japan; [2]Dept. of Pharm. and Physiol., Chulalongkorn Univ., Bangkok, Thailand; [3]Sec. Viral Vector Develop., Natl. Inst. Physiol. Sci., Okazaki, Japan; [4]Grad. Univ. of Advanced Studies (SOKENDAI), Hayama, Japan

Abstract: It is known that the superior colliculus (SC) plays a major role in the control of orienting and avoidance behaviors to visual stimulation. Although it has been reported that the crossed tecto-reticular pathway controls orienting responses and that uncrossed tecto-reticular pathway is involved in avoidance behavior, the evidence was rather indirect (Redgrave et al., 1986). It is also reported that the former neurons were distributed in the caudo-lateral portion of SC and the latter neurons in its rostro-medial area. However, the functional dissection of these two groups of neurons had been difficult. Recently, by using a combination of two viral vectors, we succeeded in selective blockade of the crossed tecto-reticular pathway in intermediate layer of SC (SGI) by expression of tetanus neurotoxin (Sooksawate et. al., 2013). In the present study, first, we injected AAV-CAG-ChR2(H134R)-tdTomato into the intermediate layer of the mouse SC(SGI). By irradiating blue laser (473nm) under freely-moving condition, we could observe the

orienting response and/or avoidance response depending on the site of stimulation in the SC. We confirmed the optogenetic activation of the SGI neurons using *in vivo* electrophysiological recordings under anesthesia. Secondly, we applied the combination of two viral vectors to selectively activate the crossed or the uncrossed tecto-reticular pathway. We injected neuron-specific highly efficient retrograde gene transfer vector (NeuRet-CMV-MSCV-U3-nls/Cre) into the brainstem reticular formation, followed by injection of the anterograde viral vector (AAV-DJ-EF1a-double flex-hChR2(E123T/T159C)-YFP) into the SGI either on the contralateral or on the ipsilateral side to the brainstem injection. The orienting movements could be observed in both groups after optogenetic activation but cringing or escape behaviors could be observed only in the case of activating the uncrossed pathway. We confirmed the activation of SGI neurons in response to the laser irradiation electrophysiologically under anesthesia, and observed differential distribution of YFP expression histologically between the crossed and uncrossed tecto-reticular neurons. Thus, by introducing the pathway-specific gene expression technique, we could dissect the function of crossed and uncrossed tecto-reticular pathways.

Disclosures: **K. Isa:** None. **T. Sooksawate:** None. **K. Kobayashi:** None. **T. Isa:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.06/S20

Topic: D.06. Eye Movements

Support: CIHR MOP-89785

Title: Resting-state functional connectivity changes following an ischemic frontal cortex stroke in a macaque

Authors: *R. ADAM[1], K. JOHNSTON[1], R. HUTCHISON[2], S. EVERLING[1];
[1]The Univ. of Western Ontario, London, ON, Canada; [2]Harvard Univ., Cambridge, MA

Abstract: Unilateral spatial neglect is a disorder of spatial attention commonly seen following right hemispheric stroke. It is characterized by an inability to attend to stimuli contralateral to the lesion, resulting in a saccade choice bias to the ipsilesional hemifield. Here, we aim to correlate the behavioural recovery of the saccade choice bias following stroke with changes in whole-brain functional connectivity (FC). We predict that increased FC within parietal areas will underlie the recovery of a saccade choice bias following a unilateral frontal cortex stroke. We created a macaque model of ischemic stroke using endothelin-1 injections to create lesions in the right dorsolateral prefrontal cortex and frontal eye field as verified by MRI. Following stroke, the animal exhibited both a profound ipsilesional saccade choice bias and increased contralesional saccadic reaction times. Resting-state fMRI scans obtained one week following stroke showed

reduced frontoparietal FC in the ipsilesional hemisphere and some increased FC between the contralesional frontal cortex and contralesional and ipsilesional posterior parietal cortex. Resting-state fMRI scans obtained four weeks following stroke showed strong increases in FC between contralesional frontal areas and both contralesional and ipsilesional posterior parietal cortex and between the contralesional and ipsilesional posterior parietal cortex. Results reveal functional reorganization following frontal cortex stroke and how changes in whole-brain FC relate to recovery of spatial neglect.

Disclosures: **R. Adam:** None. **K. Johnston:** None. **R. Hutchison:** None. **S. Everling:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.07/T1

Topic: D.06. Eye Movements

Support: F32-EY023922

T32-EY07135

R01-EY08890

R01 EY01988

P30-EY08126

P30-HD015052

Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: A premotor eye field in the arcuate sulcus of macaque monkeys - comparison with FEF

Authors: *W. ZINKE, J. D. COSMAN, G. F. WOODMAN, J. D. SCHALL;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: It is puzzling that the frontal eye field (FEF) in humans often is found in agranular area 6, while in macaques it is located within granular area 8. This different localization could be due to experimental modalities used to functionally determine FEF. In humans, the localization is primarily based on neuroimaging, whereas in monkeys it is based on microstimulation, neuronal recordings and anatomical connectivity. Several macaque neuroimaging studies report increased activation related to saccades in the posterior bank of the arcuate sulcus, consistent with the presence of a premotor eye field distinct from the FEF. Although neuronal responses to visual stimuli and with saccades were reported in the premotor cortex of monkeys, a direct comparison with FEF is still outstanding. Using linear microelectrode arrays in two macaque

monkeys (M. radiata) guided by high resolution structural MRI, neuronal activity was recorded in the anterior and posterior bank at the genu of the arcuate sulcus while the monkeys performed memory guided saccade and visual search tasks. Based on their response modulation, neurons were classified as visual, visuomovement, and movement. We identified a location at the spur of the arcuate sulcus where neurons showed clear visual responses during the tasks. The response characteristics were comparable to those of FEF neurons. We found similar proportions of neuron types in both areas, with slightly more visual neurons in the premotor area and more visuomovement neurons in FEF. We observed less transient visual responses and stronger pre-saccadic response in FEF as compared to premotor neurons. Both areas showed similar response latencies and predominantly contralateral receptive and movement fields. Our findings confirm the presence of a premotor eye field in the posterior bank of the arcuate sulcus of macaques with response properties mirroring several of those in FEF although with less saccade-related activity. The existence of functional comparable regions in nearby but cytoarchitectonical different cortical areas may explain the apparent discrepancy of FEF localization in humans and macaques.

Disclosures: W. Zinke: None. **J.D. Cosman:** None. **G.F. Woodman:** None. **J.D. Schall:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.08/T2

Topic: D.06. Eye Movements

Support: F32-EY023922

T32-EY07135

R01-EY08890

R01-EY01988

P30-EY08126

P30-HD015052

Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Comparison of saccade target selection in frontal and premotor eye fields of macaques

Authors: *J. D. COSMAN[1], W. ZINKE[2], G. WOODMAN[2], J. SCHALL[2];
[1]Dept. of Psychology, [2]Vanderbilt Univ., Nashville, TN

Abstract: The macaque frontal eye fields (FEF), located on the anterior bank of the arcuate sulcus, play a critical role in saccade target selection during visual search, with single unit responses evolving to select target locations relative to distractor locations prior to the initiation of a saccadic response. Further, such target selection is observed even in cases where a saccadic response is not required, suggesting that target selection represents a general attentional property of FEF rather than being specific to the overt allocation of attention. Importantly, similar target-selective responses have been observed in dorsal premotor cortex, posterior to the arcuate sulcus, during manual reaching tasks that require selection of a target among distractors. Interestingly, saccadic responses can be evoked in premotor cortex with low currents microstimulation, and macaque functional imaging studies have shown saccade related activation in premotor cortex. We investigated whether this putative premotor eye field is involved in both attentional selection and saccade production when saccadic but not manual target selection is required, mirroring FEF. Using linear microelectrode arrays in two macaque monkeys (M. radiata) guided by high resolution structural MRI, neuronal activity was recorded in the anterior and posterior bank at the genu of the arcuate sulcus while the monkeys performed a T/L search task in which they made saccadic responses to a target item in a field of distractors. We contrasted target selection across FEF and the premotor area, comparing the timing and magnitude of selection. Target selection was qualitatively similar between FEF and the premotor area with similar proportions of units showing target-selective responses. However, target selection occurred earlier in FEF than in the premotor area for all but the fastest reaction times. Also, response magnitude prior to the initiation of saccadic response to targets was much larger in FEF than in the premotor area.Taken together, our results suggest that both, prearcuate FEF and postarcuate premotor cortex, contribute to visuospatial attentional selection, but that selection in FEF may be more important for saccade generation on the basis of these attentional signals.

Disclosures: **J.D. Cosman:** None. **W. Zinke:** None. **G. Woodman:** None. **J. Schall:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.09/T3

Topic: D.06. Eye Movements

Support: NSERC

Title: Role of macaque dorsolateral prefrontal cortex in flexible and mnemonic attentional allocation during visual search

Authors: B. BELBECK, S. EVERLING, S. G. LOMBER, *K. D. JOHNSTON;
Univ. of Western Ontario, London, ON, Canada

Abstract: A critical aspect of visual selective attention is that it may be flexibly deployed based on the specific demands of the task at hand. Such may be the case when the features of target stimuli change frequently or when a representation of a target stimulus must be retained temporarily within working memory. Numerous lines of evidence have linked the prefrontal cortex (PFC) to cognitive flexibility and working memory, but the link between these processes and the deployment of attention has been less studied. Here, we investigated the role of the DLPFC in cognitive processes related to attentional allocation by reversibly cryogenically deactivating this area while two rhesus macaques performed a series of visual search tasks designed to vary systematically with respect to their cognitive demands. The animals performed three variants of a visual conjunction search task in which targets were defined by a unique conjunction of colour and shape. In the first, the search target was instructed at the beginning of, and remained constant throughout a given session. Correct performance of this task required that a single search target be retained throughout each session. In the second, the search target was cued at the beginning of each trial, and varied throughout a given session, requiring continuous updating of relevant target features on a trial-by-trial basis. In the third, a mnemonic demand was introduced by adding a delay period following the search target cue. In a given experimental session, the animals performed one of these tasks while the cortex lining the banks of the caudal principal sulcus was bilaterally deactivated. We observed deactivation-induced changes in task performance that varied as a function of the cognitive demands of the search task performed. During constant-target search, we observed no significant effects on performance accuracy, and inconsistent effects on reaction time. During the variable-target search task, response accuracy was unaffected, but consistent increases in reaction times were observed. The greatest effects were apparent in the variable-target delayed search task, in which both decreases in response accuracy, and increases in reaction times were found during DLPFC deactivation. These results suggest the DLPFC plays a critical role in the deployment of attention to a target when cognitive demands are high, as is the case when targets change frequently or must be maintained within working memory. These findings are consistent with the notion that the PFC contributes to attentional guidance by providing bias signals to other brain areas based on knowledge and goals of the current task.

Disclosures: **B. Belbeck:** None. **S. Everling:** None. **S.G. Lomber:** None. **K.D. Johnston:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.10/T4

Topic: D.06. Eye Movements

Support: CIHR Grant 44067

Title: Effects of iontophoretic application of muscarinic agonists on mnemonic rule representation of monkey prefrontal neurons engaged in a rule-contingent saccadic task

Authors: *A. J. MAJOR, S. VIJAYRAGHAVAN, S. EVERLING;
Western Univ., London, ON, Canada

Abstract: The dorsolateral prefrontal cortex (DLPFC) has a well-studied role in executive function, including attention, working memory (WM), and rule-contingent processing. These functions are modulated by the ascending cholinergic neuromodulatory system. Cholinergic dysfunction is thought to be a contributing factor to neurological and psychiatric disorders such as Alzheimer's disease and schizophrenia, where morbidity of prefrontal-dependent functions such as attention and WM is observed. Further, cholinergic denervation of the DLPFC in macaques or systemic pharmacological blockade of muscarinic receptors disrupts WM processing. Local muscarinic modulation of rule-contingent mnemonic processing in DLPFC has not been examined in detail hitherto. We have previously shown that the muscarinic antagonist scopolamine suppresses rule-memory in prefrontal neurons in a rule-cued pro-/antisaccade task. Two receptor classes mediate muscarinic actions: the M1 receptor family, which is densely expressed in DLPFC, predominantly postsynaptic on pyramidal spines, and the M2 receptor family, located presynaptically, but also on postsynaptic spines in projections from DLPFC area 46 to DLPFC area 9. In this study, we examined the *in vivo* effects of three iontophoretically applied cholinergic agonists on macaque DLPFC neurons: nonspecific cholinergic agonist, carbachol; M1-preferring agonist, McN-A-343; and oxotremorine-M, an orthosteric muscarinic agonist with some relative selectivity for M2 receptors *in vivo*. Preliminary data indicate that all three agonists had both excitatory and inhibitory effects on DLPFC neurons encoding rule selectivity. We found carbachol both significantly inhibited (23/54) and excited (15/54) DLPFC neurons (rule-selective: 9/18 inhibited, 3/18 excited). McN-A-343 predominantly inhibited DLPFC neurons (18/26 inhibited, 4/26 excited; rule-selective: 7/12 and 3/12, respectively), while oxotremorine-M mostly excited DLPFC neurons tested (3/16 inhibited, 10/16 excited). These are the first results to report the effect of local muscarinic stimulation to primate DLPFC rule-contingent WM circuits, and support muscarinic receptors as pharmacological targets for treatment of cognitive deficits.

Disclosures: A.J. Major: None. **S. Vijayraghavan:** None. **S. Everling:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.11/T5

Topic: D.06. Eye Movements

Support: The Strategic Research Program for Brain Sciences, “Development of BMI Technologies for Clinical Application” and “Construction of System for Spread of Primate Model Animals”, by the Japan Agency for Medical Research and Development (AMED)

Precursory Research for Embryonic Science and Technology (PRESTO) from the Japan Science and Technology Agency

Grants-in-aid for scientific research (26120715) from the Japan Society for the Promotion of Science

Title: Analysis of eye movements after repetitive transcranial magnetic stimulation on behaving monkeys

Authors: *K.-I. OKADA[1,2], Y. KOBAYASHI[1,2,3,4];
[1]Osaka Univ., Suita, Japan; [2]Ctr. for Information and Neural Networks (CiNet), Natl. Inst. of Information and Communications Technology, and Osaka Univ., Osaka, Japan; [3]Osaka Univ. Res. Ctr. for Behavioral Econ., Suita, Japan; [4]PRESTO, Japan Sci. and Technol. Agency (JST), Saitama, Japan

Abstract: Transcranial magnetic stimulation (TMS) is a technique for non-invasive brain stimulation that uses a high-intensity and brief electromagnetic induction to generate intracranial currents. Repetitive application of TMS pulse (rTMS) has emerged as a potential treatment option for several neurological disorders, including depression, neuropathic pain, and Parkinson’s disease (PD). PD is a type of movement disorder that causes a loss of dopaminergic neurons in the basal ganglia. In the treatment for PD, rTMS over the primary motor cortex, supplementary motor area, and dorsolateral prefrontal cortex at 1-10 Hz has been used. As the main pathological of PD is the death of dopaminergic neurons in the basal ganglia, cortical stimulation by rTMS somehow affects the function of large scale neural circuit including the cortical or subcortical regions. Exactly, previous imaging study using positron emission tomography reported that rTMS over the motor cortex induces a release of endogenous dopamine in the ventral striatum in humans and monkeys. Furthermore, study of functional magnetic resonance imaging for PD patients reported that the improvement in bradykinesia by rTMS is associated with activity increase in caudate nucleus. However, despite widespread use of rTMS, exactly how rTMS influences neural activity throughout an interconnected network, and how such influences ultimately change disease symptoms, remain unclear. Appropriate animal model is required to understand how rTMS influences large scale neural circuit and behavior. One potential model system is the oculomotor system of monkeys that is the one of the most well-established system of the brain, which include both cortical areas and basal ganglia nuclei, and contribution of each region to saccadic behavior is well characterized. Here we tested the effect of rTMS to oculomotor behavior in monkeys. In order to record eye movement and set TMS coil precisely and reproducibly, monkeys head were fixed by thermoplastic mask and cheek rest. rTMS were applied using a MagStim Rapid Transcranial Magnetic Stimulator with a figure-eight coil. Left and right eye positions and pupil size of monkeys were acquired with a fast video-based eye movement monitor (Eyelink1000).

Disclosures: **K. Okada:** None. **Y. Kobayashi:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.12/T6

Topic: D.06. Eye Movements

Title: Neural activation and saccadic eye movements involved during letter and object naming speed tasks

Authors: *N. Z. AL DAHHAN, D. C. BRIEN, J. R. KIRBY, D. P. MUNOZ;
Queen's Univ. Ctr. for Neurosci. Studies, Kingston, ON, Canada

Abstract: Naming speed (NS) tasks, which measure how quickly and accurately subjects can name sets of highly familiar stimuli (e.g., letters) randomly presented in a visual array, have been shown to be a precursor and concurrent correlate of accurate and efficient reading. However, it is still not known what cognitive processes underlie this relationship. Functional magnetic resonance imaging (fMRI) was used to investigate the neural substrates and cognitive processes underlying performance during letter and object NS tasks. We used three methods to examine task performance: (a) changing stimulus composition to emphasize phonological and/or visual aspects; (b) decomposing NS times into pause and articulation components; and (c) analyzing eye movements and brain activation involved in a NS task. We recruited 19 healthy young adults (ages 21 - 26 years), and employed a block design consisting of a letter NS task and three variants that were either phonologically and/or visually confusing (Compton, 2003); and an object NS task with a variant in which the object names rhymed with one another, while subjects' eye movements and articulations were recorded. We examined how these manipulations influenced performance and whether they resulted in differences in neural activation. Behavioral analyses revealed that for the letter NS task, NS manipulations were associated with specific patterns of performance which were influenced by visual rather than phonological similarity. When the task was both visually and phonologically similar, participants had significantly longer naming times and fixation durations, and made more frequent saccades and regressions. However, for the object NS tasks participants' performance was not affected when the names of the objects rhymed with one another. This was indicated by a trend in which participants made shorter fixations and fewer saccades, and had significantly shorter naming times and made fewer regressions on the phonologically similar object task than the control task. fMRI results indicated significant activation during both letter and object NS tasks in brain areas involved in the reading network and in tasks that require eye movement control and attention in typical adult readers, such as the temporo-parietal area, inferior frontal cortex, superior temporal gyrus, and the ventral visual stream. Further analyses revealed that the different task manipulations target key structures within this reading network, such as the anterior cingulate cortex, precuneus, middle temporal gyrus, and thalamus. These findings reveal that NS tasks recruit the same network of neural structures that are involved in reading.

Disclosures: **N.Z. Al Dahhan:** None. **D.C. Brien:** None. **J.R. Kirby:** None. **D.P. Munoz:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.13/T7

Topic: D.06. Eye Movements

Title: Concurrent and directionally-compatible hand movements reduce fixation durations and increase peak velocity of sequential saccades

Authors: *B. PARSONS, R. IVRY;
UC Berkeley, Berkeley, CA

Abstract: Studies looking at the relationship between concurrent manual and ocular movements have tended to focus on the influence that gaze has on subsequent hand movements. When responding to a visual cue, the saccade reaction time and duration is faster than the manual response. Indeed, the saccade is typically completed before the reaching movement begins and the saccade may influence the metrics and kinematics of the arm movement. The reverse influence of manual movements on the saccade remains relatively unexplored. Studies that have looked at the phenomenon mostly report a delay in the initiation of saccades when concurrent hand movements are planned. We present evidence that during sequences of horizontal saccades, the maximum rate of the eye movements increases when the eye movements are accompanied by hand movements. This increase is the result of both shorter fixation durations and higher peak velocity. Changes in peak velocity and fixation duration were observed both in the presence and absence of saccadic inhibition of return (Hooge & Frens 2000), although the size of the effect is diminished in the latter condition. The saccade facilitation from arm movements requires that the two actions are directionally compatibility. When the hand moves in the direction opposite to the saccade, fixation durations are longer than in a saccade-only condition. Auditory cueing of saccade sequences produced a modest reduction in fixation duration, arguing against a purely attentional account of the effect. These results provide novel insight into the temporal coordination between saccade and arm motor systems during sequential movements.

Disclosures: **B. Parsons:** None. **R. Ivry:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.14/T8

Topic: D.06. Eye Movements

Support: CIHR 9222

Title: Refuting the hypothesis that a unilateral human parietal lesion impairs saccade corollary discharge

Authors: *K. RATH-WILSON, D. GUITTON;
Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: This study questions the current dominant hypothesis suggesting an impairment of saccade-related corollary discharges (CD) in patients with unilateral parietal lesions, especially of the right hemisphere. A CD is an efferent copy of the motor command for a saccadic eye movement which is distributed to various sensorimotor areas of the brain to update perceptual and motor systems about self-generated movements. In the classic double-step saccade task, used to investigate saccade-related CDs, two targets (T1 and T2) are briefly (80-140ms) flashed sequentially in the periphery. With the extinction of the fixation point and targets, subjects are asked to make two saccades, in the dark, to the remembered locations of the targets in the order they appeared (S1 to T1, S2 to T2). Duhamel et al (1992) and Heide et al (1995) argued that patients with a unilateral parietal lesion fail at generating an accurate second saccade when the first one is directed contralesionally, but not ipsilesionally, suggesting an impairment in contralesional CD generation. Here, we show first that this conclusion is oversimplified. We tested 5 parietal patients on a classic double step task. When given sufficient time and when corrective saccades were evaluated: 2 of 5 patients produced a CD for first saccades in either direction; 2 other patients produced a CD for first saccades only in the contralesional direction; and the last patient did not show evidence of CD. We hypothesize that the continued difficulties of some patients to successfully complete the task relates to well-described attentional impairments in parietal patients. We then tested the same 5 plus another parietal patient (4 left, 2 right) on a modified version of the double step task, designed to mitigate some of the common attentional deficits associated with parietal lesions. We presented T2 first for a longer period of time (800-1200ms) and then T1 (350ms). Patients, in the dark, were to look at where T1 had just appeared and then to the remembered location of T2. All patients who completed sufficient trials showed evidence of CD for ipsilesional and contralesional saccades on this task. Next, in a new task, a single target (T) was flashed and the patients had to first make a self-generated, “endogenous”, saccade of self-determined amplitude before making a second saccade to the remembered location of the previously flashed T. All patients used CD for both ipsilesional and contralesional first saccades on this task. Thus, contrary to current thinking, unilateral left, or right, parietal patients could produce a CD for both left and right saccades.

Disclosures: K. Rath-Wilson: None. **D. Guitton:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.15/T9

Topic: D.06. Eye Movements

Support: NSERC

CIHR

Title: A six-month exercise-training program improves cognitive-motor control in persons with an identified cognitive complaint: Evidence from the antisaccade task

Authors: ***M. D. HEATH**, C. GILLEN, J. WEILER, M. GREGORY, D. GILL, R. PETRELLA; Univ. of Western Ontario, London, ON, Canada

Abstract: The incidence of individuals reporting a subjective cognitive complaint or demonstrating objective cognitive deficits (i.e., cognitive impairment, not dementia; CIND) is twofold greater than that for Alzheimer's disease (AD) and related dementias. Recent work by our group has shown that a six-month targeted exercise intervention program in persons with CIND improves general cognitive performance and may decrease the risk for further cognitive decline. The goal of the present investigation was to determine whether an exercise intervention impacts goal-directed eye movements in persons with an identified cognitive impairment - a question that provides a framework to determine whether cognitive impairment and exercise influence the basic cognitive-motor processes underlying activities of daily living. Individuals with objectively measured cognitive impairment (Montreal Cognitive Assessment scores < 26) but not dementia (Mini Mental State Examination scores > 24) and their age- and sex-matched controls (individuals with no objectively measured cognitive impairment) participated in a six-month exercise intervention involving dual-task gait training and moderate intensity aerobic exercise on a specialized treadmill (30 minutes, 3 times/week). Prior to and following the intervention, participants completed goal-directed eye movements requiring responses to the veridical location of a target stimulus (i.e., prosaccade) and to the target's mirror-symmetrical location (i.e., antisaccades). In particular, we contrasted pro- and antisaccades because the latter task is cognitively demanding and is supported via cortical structures that have been linked to cognitive decline in dementia (i.e., dorsolateral prefrontal cortex). As expected, prosaccade reaction times (RT) were shorter than antisaccades. More notably, antisaccades - but not prosaccades - for persons with a cognitive impairment showed a selective decrease in RTs post-intervention. As such, we provide evidence that exercise can improve cognitive-motor function in persons with cognitive impairment. As well, our results suggest that a targeted exercise intervention program may serve to attenuate adverse structural changes to those cortical regions related to dementia.

Disclosures: **M.D. Heath:** None. **C. Gillen:** None. **J. Weiler:** None. **M. Gregory:** None. **D. Gill:** None. **R. Petrella:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.16/T10

Topic: D.06. Eye Movements

Title: Effects of flight duration, expertise, and arousal on eye movements in aviators

Authors: *A. M. MELCHIADES NOZIMA[1], L. L. DI STASI[2], S. MARTINEZ-CONDE[3], M. MCCAMY[4], E. GAYLES[5], A. G. COLE[6], M. J. FORSTER[6], B. HOARE[6], F. TENORE[7], M. JESSEE[7], E. POHLMEYER[7], M. CHEVILLET[7], A. CATENA[2], W. C. DE SOUZA[8], S. L. MACKNIK[3];
[1]Adriana Nozima, New York, NY; [2]Mind, Brain, and Behavior Res. Ctr. Univ. of Granada, Granada, Spain; [3]Ophthalmology, Neurology, and Physiology/Pharmacology, State Univ. of New York, New York, NY; [4]Barrow Neurolog. Inst., Phoenix, AZ; [5]Third Marine Aircraft Wing Marine Corps Air Station Miramar, Camp Pendleton, CA; [6]3D Marine Air Wing (MAW), Marine Aviation Training Syst. Site (MATSS), Camp Pendleton, CA; [7]Biol. Sci. and Engin. Group, Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; [8]Univ. de Brasilia, Brasilia, Brazil

Abstract: Eye movements can reveal where, what, why, and how the human brain processes the constant flow of visual information from the world. Here, we measured the eye movements of United States Marine Corps (USMC) combat aviators to understand the oculomotor effects of fatigue as a function of time-on-flight (TOF), expertise, and arousal during flight training. Saccadic velocities decreased after flights lasting 1h or more, suggesting that saccadic velocity could serve as a biomarker of aviator fatigue. We observed that USMC aviators can be significantly fatigued by simulator training. A follow-on study set out to determine, via oculomotor measures, if TOF affected the aviators' cognitive processes. To test this we examined oculomotor dynamics in response to emergency procedures in flight, and found that the effects of TOF on eye movements were alleviated temporarily by the addition of high-arousal stressful conditions. Finally, we tested whether novice pilots might benefit from passive viewing of expert eye position scanpaths. We tasked novice pilots with repeatedly resolving a serious emergency procedure (dual engine failure cascade), followed by watching a video with an expert solving the same emergency procedures: half of the novices saw the video with the expert eye position indicated, and the other half watched the video without eye movements superimposed. Pilots who were given the expert eye movement information performed better in a subsequent test, and specifically incorporated eye movement strategies from the expert in their behavior.

Disclosures: **A.M. Melchiades Nozima:** None. **L.L. Di Stasi:** None. **S. Martinez-Conde:** None. **M. Mccamy:** None. **E. Gayles:** None. **A.G. Cole:** None. **M.J. Forster:** None. **B. Hoare:**

None. **F. Tenore:** None. **M. Jessee:** None. **E. Pohlmeyer:** None. **M. Chevillet:** None. **A. Catena:** None. **W.C. de Souza:** None. **S.L. Macknik:** None.

Poster

334. Eye Movements: Neurophysiology of Saccades

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 334.17/T11

Topic: D.06. Eye Movements

Support: NIH Grant R01EY024844

Title: Modulation of eye movements by down syndrome cell adhesion molecule-like 1

Authors: *M. M. MA, Y. A. PAN;
Dept. of Neurosci. and Regenerative Med., Georgia Regents Univ., Augusta, GA

Abstract: Normal brain function requires precise wiring of neuronal connections, established during gestation and childhood periods. Recent genetic studies of autism spectrum disorder (ASD) families have identified the Down syndrome cell adhesion molecule (DSCAM) and its close homolog, DSCAM-Like1 (DSCAML1), as potential causal genes for the disease. DSCAM and DSCAML1 are membrane adhesion molecules known to regulate dendritic outgrowth and selective cellular adhesion. It is still unclear, however, how DSCAM and DSCAML1 affect broader brain connectivity and behavior. To address these questions, we examined the role of DSCAML1 in visual function, using larval zebrafish, a small and transparent vertebrate, as the model. We generated a putative null allele in the zebrafish DSCAML1 gene by TALEN-directed mutagenesis. Mutant animals are grossly morphologically normal but never survive past 10 days post-fertilization. As DSCAML1 has previously been shown to affect development of the visual system, specifically the retina and its afferents, we tested whether loss of DSCAML1 affects an innate visual behavior, the optokinetic reflex (OKR). OKR involves eye movements in response to whole-field motion stimulus in the surroundings, which stabilizes the visual image on the retina. OKR is conserved among vertebrates and serves as a robust assay to investigate sensory processing, motor control, and diagnosis of brain lesions or abnormal development. In contrast to control, which can initiate OKR within the first two trials, DSCAML1 mutants required three or more trials to initiate OKR. Interestingly, once initiated, there was no significant difference between the mutants and the controls in OKR performance (speed over different contrast levels). In addition, mutant animals exhibited deficits in conjugated eye movements. These results suggest that DSCAML1 is required for establishing the neural circuitry for OKR, specifically the initiation and coordination of eye movements. The higher threshold for initiating OKR may potentially result from deficits in a gating mechanism within the mid-hindbrain OKR neural circuitry. These findings may be clinically relevant to the eye movements disorders seen in

individuals with ASD. Brain mapping analyses with transsynaptic vesicular stomatitis virus (VSV) are currently underway to identify the neural substrates for the OKR deficits.

Disclosures: M.M. Ma: None. **Y.A. Pan:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.01/T12

Topic: D.07. Vestibular System

Support: RFFI Grant 13-04-01736

Title: Compensatory changes of tetha oscillations in hippocampus after vestibular sensory loss in rats

Authors: *J. N. ERON, N. A. LOGINOVA, V. A. KORSHUNOV;
Inst. of Higher Nervous Activity and Neurophysiol., Moscow, Russian Federation

Abstract: The theta oscillations in hippocampus synchronize firing rate of hippocampal cells, which appear to encode an animal's location within the environment, and also play an essential role in spatial orientation and memory. It has been shown that vestibular sensory damage causes the reduction of theta rhythm in hippocampus and produces long-term changes in performance in a spatial reference memory tasks. Nevertheless, it is unknown whether the short-term compensatory changes of theta oscillations occur when the vestibular sensory input is completely loss. The aim of this study was to estimate the short-term changes of theta activity due to vestibular sensory loss. The local field potential oscillations were recorded in CA1 hippocampus in awaked freely moving animals (male Wistar rats, age >3 months) to estimate the phase-amplitude coupling of theta band rhythm. The rhythmic activity were tested before and again in 2, 4-6, 8, 10-12, 15 and 20-30 days after bilateral vestibular deafferentation (BVD) (n=7) or sham vestibular loss (n=7). After BVL the theta rhythm frequency shifted from 7-9 Hz to 4 Hz and the power significantly decreased; however, significant temporal recovery of the frequency range and power of theta rhythm was found by post-BVD day 12. There were no significant changes in spectral properties of theta oscillations in CA1 hippocampus in rats after the sham vestibular loss. Thus, the facilitation of theta oscillations in hippocampus was observed post-BVD term. Recently, we have reported about similar short-term recovery of synaptic transmission efficacy in CA1 hippocampus after BVD. Our results suppose that the facilitation of theta oscillations and synaptic transmission could occur due to increase of excitability in neuronal circuits, which could be associated with synchronization of neuronal responses in hippocampus during relearning to new inter-sensory interaction, when the vestibular sensory input is damaged.

Disclosures: **J.N. Eron:** None. **N.A. Loginova:** None. **V.A. Korshunov:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.02/T13

Topic: D.07. Vestibular System

Support: Pierre and Marie Curie Staff Exchange Programme IRSES (FP7-PEOPLE-2012-IRSES (grant agreement number 318980 - SVETA)

Centre national d'études spatiales (DAR 2012-2013)

Title: Effects of vestibular loss and parabolic flight on cell proliferation in the rat dentate gyrus

Authors: ***C. M. GLIDDON**[1,2], Y. ZHENG[2], P. AITKEN[2], L. STILES[2], M. HITIER[3], M.-L. MACHADO[4], B. PHILOXENE[4], P. DENISE[4], P. SMITH[2], C. DARLINGTON[2], S. BESNARD[4]; [1]Pharmacol. and Toxicology Dept., Univ. of Otago, Dunedin, New Zealand; [2]Brain Hlth. Res. Centre, Pharmacol. and Toxicology Dept., Dunedin, New Zealand; [3]Dept. of Otolaryngology Head and Neck Surgery, Caen, France; [4]Normandie Univ., Caen,, France

Abstract: There is evidence to suggest that alterations in gravity as experienced by astronauts can induce impairment in spatial learning and memory; however, the underlying mechanism remains to be elucidated. Dysfunction of the vestibular apparatus, which detects gravitational acceleration, has been linked to hippocampal malfunction and spatial learning and memory impairment. One possibility is that the impairment of spatial learning and memory following the alteration in gravity is due to changes in cell proliferation and neurogenesis within the hippocampal dentate gyrus, a well-known neurogenic area. The aim of this experiment was to determine if changes in gravity during parabolic flight, altered cell proliferation in the dentate gyrus of rats with either an intact vestibular apparatus (sham group) or no vestibular apparatus (bilateral surgical vestibular deafferentation, BVD) by measuring the number of bromodeoxyuridine (BrdU)-labelled cells. Male Long-Evans rats were randomly allocated to the following experiment groups: (1) sham surgery only (n = 5); (2) BVD surgery only (n = 5); (3) sham surgery and parabolic flight (n = 5); (4) BVD and parabolic flight (n = 6). At 6 weeks following the surgery, the animals were subjected to parabolic flight under anaesthesia (fentanyl, 0.2 mg/kg, s.c. and medetomidine hydrochloride, 0.5 mg/kg, s.c.). The parabolic flight was carried out on a modified Airbus A300 aircraft. The no flight animals were under the same anaesthesia for the same duration. Immediately after the parabolic flight, all animals were injected with BrdU (300 mg/kg, i.p). Twenty-four hours after BrdU injection, rats were anaesthetized and cardiac-perfused with saline followed by 4% paraformaldehyde. The brains were dissected out. Serial 40 μm sagittal sections throughout the hippocampus were cut

according to a random, systematic sampling design. BrdU immunolabelling was performed and the number of BrdU positive cells was quantified using a modified fractionator method. Data were analysed using a linear mixed model analysis in SPSS 22. There was a significant main effect of BVD ($F(1, 34) = 15.21$, $P \leq 0.0001$); however, side, flight, and all interactions were non-significant. The results indicate that BVD significantly decreased the number of BrdU-positive cells compared to sham-operated animals. However, brief cyclic periods of hypergravity and microgravity induced by a parabolic flight does not significantly alter the number of BrdU-positive cells in either sham or BVD rats, suggesting that changes in gravity do not alter the proliferation of cells within the dentate gyrus.

Disclosures: **C.M. Gliddon:** None. **Y. Zheng:** None. **P. Aitken:** None. **L. Stiles:** None. **M. Hitier:** None. **M. Machado:** None. **B. Philoxene:** None. **P. Denise:** None. **P. Smith:** None. **C. Darlington:** None. **S. Besnard:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.03/T14

Topic: D.07. Vestibular System

Support: NIH Grant DC012630

Title: Otolithic involvement in the organization of exploratory movements

Authors: L. A. CHEREP[1], P. A. BLANKENSHIP[1], S. N. BROCKMAN[3], A. D. TRAINER[3], J. D. BENSON[2], *R. M. YODER[3], D. G. WALLACE[1];
[1]Dept. of Psychology, [2]Dept. of Mathematical Sci., Northern Illinois Univ., DeKalb, IL; [3]Dept. of Psychology, Indiana Purdue Fort Wayne, Fort Wayne, IN

Abstract: Rodents use environmental and self-movement cues to organize their exploratory behavior. A portion of these self-movement cues appears to include signals from the otolith organs, given the marked path integration deficits of otoconia-deficient mice on a food-hoarding task in darkness. Access to environmental cues improves performance on this task in light, but some aspects of performance are still impaired. These deficits are not fully understood at the present time, but may involve disruptions in movement organization; however, no previous studies have evaluated the otolithic contribution to exploratory behavior. The organization of exploratory behavior was characterized in otoconia-deficient *tilted* mice when they moved about an open field under dark and light conditions. The open field was a circular table (d = 112 cm) with a transparent wall (15 x 20 cm) affixed to the edge that could serve as a polarizing tactile cue. An overhead video camera recorded all movements for offline analysis. Each mouse was released at the center of the arena and permitted to explore the entire apparatus for one 40-min

trial each day, across three days in darkness and three days in light. Movement characteristics were examined after the first bout of grooming. Exploratory paths were divided into stops and progressions. Several measures were developed to evaluate stops - number of stops, stop duration, change in heading, and locations of stops. Several measures were developed to evaluate progressions - number of progressions, peak speed, path circuity, and path length. In general, both groups of mice groomed and established a "home base" near the location of grooming in both darkness and in light. In darkness, but not in light, the concentration of stops was lower for *tilted* mice than control mice. Also in darkness, but not in light, *tilted* mice showed more circuitous progressions than control mice. Interestingly, *tilted* mice showed a greater change in heading after stops, in both darkness and in light. This pattern of results is consistent with impaired self-movement cue processing in the absence of signals from the otolith organs. Along with our previous studies, these results indicate an important role for otolithic information in the organization of exploratory movements.

Disclosures: L.A. Cherep: None. **P.A. Blankenship:** None. **S.N. Brockman:** None. **A.D. Trainer:** None. **J.D. Benson:** None. **R.M. Yoder:** None. **D.G. Wallace:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.04/T15

Topic: D.07. Vestibular System

Support: CIHR

NIH

Title: Galvanic vestibular stimulation in primates: Recording vestibular afferents during transmastoid stimulation

Authors: *A. KWAN[1], D. E. MITCHELL[2], P. A. FORBES[3,4], J.-S. BLOUIN[4], K. E. CULLEN[2];
[1]Biomed. Engin., [2]Physiol., McGill Univ., Montreal, QC, Canada; [3]Biomechanical Engin., Delft Univ. of Technol., Delft, Netherlands; [4]Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Every day the vestibular system detects head motion and provides the brain with information needed for our sense of balance. The vestibular sensors in the inner ear convert this head motion information into a neural signal, which is then carried by the primary vestibular afferents to the central nervous system. In the absence of actual motion, vestibular afferents can be artificially modulated by delivering electrical current in proximity of the inner ear, termed galvanic vestibular stimulation (GVS). GVS has become a popular tool to study human

vestibular function because unlike natural vestibular stimuli requiring physical motion, it generates vestibular signals without activating other sensory channels. GVS, which is applied to human subjects between surface electrodes on the mastoid processes behind the ears, induces ocular and postural responses, and virtual motion perception, attributed to the activation of the vestibular system. While there is growing interest in the applications of GVS in biomedical research and rehabilitation, to date, it is not fully understood how GVS activates the vestibular system. Although vestibular afferent responses have been recorded during electrical stimulation, these studies used stimulating electrodes implanted in the ear. Here, to better understand how transmastoid GVS affects vestibular nerve activity, we recorded vestibular afferent responses during GVS applied between surface electrodes on the mastoid processes of alert macaques. Stimulation protocols included constant current, typically used in human GVS studies, as well as single sinusoidal and band-limited noise current corresponding to the physiological relevant frequency range (0 - 25Hz). We found that GVS activates non-specifically all vestibular afferents, both semicircular canals and otoliths, with irregular afferents being more sensitive, consistent with previous studies. We show for the first time that during sinusoidal stimulation, otolith afferents, much like canal afferents, displayed an increase in both gain and phase lead as a function of frequency. These results contrast with previous studies using internal electrodes, which instead observed a flat frequency response (i.e. relatively constant gain and phase). While comparing the responses to noise and sinusoidal stimulation suggests that the afferents encode GVS linearly, surprisingly, the afferent responses to constant current GVS could not be predicted from the model fit to sinusoidal data. Specifically, the observed time constants were longer than estimated. Together, these results provide new insights into modelling the dynamics of GVS activation of the vestibular system.

Disclosures: A. Kwan: None. **D.E. Mitchell:** None. **P.A. Forbes:** None. **J. Blouin:** None. **K.E. Cullen:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.05/T16

Topic: D.07. Vestibular System

Title: Effects of galvanic vestibular stimulation on vestibular cortex activity

Authors: *F. NOOHIBEZANJANI[1], C. KINNAIRD[1], S. WOOD[2], J. BLOOMBERG[3], A. MULAVARA[4], R. SEIDLER[1];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Col. of Liberal Arts and Sci., Azusa Pacific Univ., Azusa, CA; [3]NASA Johnson Space Ctr., Houston, TX; [4]Universities Space Res. Assn., Houston, TX

Abstract: Falls are the current leading cause of fatal and nonfatal injuries in older adults. The annual rate of falling is approximately one out of three in older adults above 65, and its direct medical cost is estimated as $34 billion. The aim of this study was to investigate the effects of Galvanic Vestibular Stimulation (GVS) on improving balance. GVS refers to the application of low-level electrical noise via electrodes placed on the mastoid bones. The addition of low-level noise to the sensory system increases the signal to noise ratio and results in more sensitivity to detecting the sensory signals. We assessed the effects of short-term and long-term exposure to GVS on brain activation and postural control. We hypothesized that low-level GVS increases vestibular cortex activity, which correlates with improvement in balance control. Further, we hypothesized that long-term GVS application leads to greater functional and anatomical alterations in the brain than short-term GVS. Subjects underwent three fMRI scans over the course of 6 days: baseline (day1), short-term effects (day2), and long-term effects (day6). We assessed the alterations in body sway on a force platform on day1 and day6 (i.e. pre and post intervention balance assessments). The GVS was applied for 4 days (days 2-5), 45 minutes per day. Since GVS increases the perception of sensory input, we evaluated subjects' threshold for signal detection prior to GVS application on each day, and modified the stimulation level accordingly. Using this method we were able to control for individual differences and day-to-day variability. The preliminary results suggest that short-term GVS effectively changes the functional activity of the vestibular cortex and other brain regions involved in sensorimotor processing. We found an overall decrease in activation of parietal-temporal regions (more directly involved with processing of vestibular input), along with increased activity of pre and post central gyri, and occipital regions (more directly involved in processing of visual and proprioceptive signals). These findings suggest that application of GVS could potentially elevate the sensitivity and function of the vestibular system in a way that there is less cortical allocation needed for processing the vestibular information, leaving the other regions involved in processing sense of motion with more resources to respond to vestibular stimulation. Our future analyses will determine the long-term effects of GVS and the extent to which the alterations in cortical activity correlate with balance improvements.

Disclosures: F. Noohibezanjani: None. **C. Kinnaird:** None. **S. Wood:** None. **J. Bloomberg:** None. **A. Mulavara:** None. **R. Seidler:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.06/T17

Topic: D.07. Vestibular System

Support: EU-ERC 283567

Title: EEG spectral signatures of spatial updating processes during whole-body motion

Authors: *T. P. GUTTELING, W. P. MEDENDORP;
Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: When moving around in the world, everything seems stable, although some objects may move along with you ('body-fixed'), while some stay fixed in the world ('world-fixed'). The latter has been studied in great detail during saccadic eye movements, revealing the existence of spatial updating mechanisms. Recently, we studied the role of such updating mechanisms during passive whole-body motion, where vestibular information is likely to drive the update. Using EEG, we found that world-fixed targets are dynamically updated, and are represented by lateralized alpha desynchronizations in parieto-occipital areas. While this shows an interaction between vestibular motion signals and dynamic updating, it is still unclear how their interaction comes about. Here we used a parametric design to examine this interaction between self-motion, target coding and spatial updating. Subjects were linearly accelerated using a translation sled, while 88-channel EEG and eye position were recorded. While subjects maintained body-fixed fixation, a peripheral target location was briefly flashed, which had to be either maintained or updated during the motion, depending on whether the target was world-fixed or body-fixed. After the motion, subjects had to indicate where the remembered target was located in space. Preliminary results show that self-motion processing, target coding and successful spatial updating are reflected in distinct oscillatory signatures that evolve during self-motion. Ongoing analyses and experiments aim to reveal the interplay between these processes that drive spatial updating, as well as manipulate ongoing cortical oscillatory processes using transcranial alternating current stimulation.

Disclosures: T.P. Gutteling: None. **W.P. Medendorp:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.07/T18

Topic: D.07. Vestibular System

Title: Robotic assessment of wrist position sense in a 3d workspace

Authors: *F. MARINI[1], V. SQUERI[1], P. MORASSO[1], L. MASIA[2];
[1]Inst. Italiano Di Tecnologia, Genova, Italy; [2]nanyang technological university, Singapore, Singapore

Abstract: The ability of perceiving of our body position in space is an important component of proprioception. It plays a crucial role in human movement control, which is fundamental for every daily activity. The mechanisms underlying proprioception have been extensively

investigated and an increasing emphasis has been placed on quantitative assessment of proprioceptive acuity. One test that has garnered particular interest is joint position matching, in which participants must replicate a reference joint angle using just proprioceptive information (in the absence of vision). Although the assessment of joint position sense has become a common topic of research, no standard method for measuring it has been established and universally accepted in clinical practice. Recent advances in robotic interfaces designed for sensorimotor rehabilitation enabled the use of such devices for the assessment of proprioceptive functions. Just few studies focused on the wrist joint, and most of them examined just a single joint degree of freedom. With the aim of assessing wrist proprioceptive acuity we designed a joint position matching task that thirty healthy adults performed with a 3 degrees of freedom wrist exoskeleton. Specifically, we sought to characterize each degree of freedom (DoF) of the joint (Flexion/extension, radial/ulnar deviation, pronation/supination) in term of its position acuity, measured as the error made while actively replicating a reference joint angle. Besides the investigation of the isotropy (or anisotropy) of the wrist position sense across its DoFs, we wanted to study if our proprioceptive acuity changes if experienced targets are located in a smaller workspace meaning that we perceive differently targets which are closer to the center of the joint. Moreover, we examined whether the distance is remembered better or worse than the final position or, in other words, if distance cues are reliable as location cues and if matching errors could depend on the use of distance information rather than just on the end point information. What we found is a significant difference in the proprioceptive acuity of the three DoFs in particular for the radial/ulnar deviation, for which we found smaller positioning errors. Furthermore, we found a consistent higher accuracy in matching targets located in a larger workspace, closer to the limits of wrist's range of motion since greater errors were observed in the small workspace condition were targets were always overestimated. Finally, we could evaluate how much subjects rely on target distance as well as its position since subjects' performance was characterized by a higher variability when the distance information was missing.

Disclosures: **F. Marini:** None. **V. Squeri:** None. **P. Morasso:** None. **L. Masia:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.08/T19

Topic: D.07. Vestibular System

Support: EU-FP7-FET SpaceCog Grant 600785

Title: Multiple spatial representations are updated in parallel during self-motion

Authors: *J. J. TRAMPER, P. MEDENDORP;
Radboud University, Donders Inst., Nijmegen, Netherlands

Abstract: The brain encodes visual space in multiple spatial representations, including eye-centered and body-centered reference frames. When we move our body in space, these internal, egocentric representations are no longer in register with external space, unless they are actively updated. Whether the brain updates multiple spatial representations in parallel, or whether it pertains its updating mechanisms to a single reference frame from which other representations are constructed, remains an open question. We developed an optimal integration model to simulate the update of a visual target across body motion in multiple or single reference frames, assuming a biased estimate of body displacement and a noisy estimate of the sensory signals. To test this model, we designed an experiment in which participants had to remember the location of a briefly presented target, while being translated sideways, in complete darkness. The behavioral responses were in agreement with a model that uses a combination of eye- and body-centered representations, weighted according to the reliability in which the target location is stored, and updated in each reference frame. Our findings suggest that the brain simultaneously updates multiple spatial representations across body motion. Because both representations are kept in sync, they can be optimally combined to provide a more precise estimate of object locations in space than based on single-frame updating mechanisms.

Disclosures: J.J. Tramper: None. **P. Medendorp:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.09/T20

Topic: D.07. Vestibular System

Support: NSERC

Canadian Chiropractic Research Foundation

Title: Spatial transformation of the vestibular control of standing in humans

Authors: P. FORBES[1], B. L. LUU[2], M. VAN DER LOOS[1], E. A. CROFT[1], J. INGLIS[1], *J.-S. BLOUIN[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Neurosci. Australia, Sydney, Australia

Abstract: The vestibular control of standing is tightly coupled to head orientation. The evoked muscles response must undergo spatial transformation to provide effective compensation and error correction. In this study we investigate two properties of this transformation and their effect on the vestibular evoked muscle response: 1) the effect of head position when the body is

constrained to a single anterior-posterior plane of motion, and 2) the effect of reversing the spatial relationship between the motor output (i.e. ankle moment) and the accompanying vestibular consequences (i.e., head motion). By using a robotic platform that simulates the mechanics of standing balance as an inverted pendulum, we can control the spatial properties of the motor signals and the subsequent vestibular sensory information. Subjects were exposed to binaural bipolar electrical vestibular stimulation applied to the mastoid processes while recording EMG from the right soleus. To address the effects of head position, subjects balanced the robotic platform under normal conditions with the head facing forward and with the head turned to the left at 15° increments up to 90°. To address the effects of spatial relationships between motor and sensory signals, subjects balanced the robotic platform with the head turned left while the torque output that generates natural body sway was reversed. We isolated the sensorimotor reversal to vestibular and motor signals. Across head rotations, the largest muscle response to vestibular stimulation was observed when the head was turned 90 degrees to the left, with the response diminishing as the head was turned towards a forward facing position. The change in peak-to-peak amplitude of the muscle response at intermediate head angles was described by the cosine transformation of the muscle response obtained with the head rotated 90 degrees. During vestibular sensorimotor reversals, the muscle response was reversed and delayed by about 30 msec in comparison to normal conditions, even though the postural sway remained in the direction expected of the electrical stimulus (i.e. sway towards the anode). The present study describes two key factors in the spatial transformation of the muscle response to electrical vestibular stimulation. First, that only the vestibular-motor pathways that are appropriate to counteract the electrically-induced sway are recruited as part of the balance response rather than a generalised response to all dependent postural muscles. And second, that the counteracting muscle response can be modified to account for changes in the spatial sensorimotor relationship but is accompanied with an increased processing delay.

Disclosures: **P. Forbes:** None. **B.L. Luu:** None. **M. Van der Loos:** None. **E.A. Croft:** None. **J. Inglis:** None. **J. Blouin:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.10/U1

Topic: D.07. Vestibular System

Support: National Health and Medical Research Council Australia G1058056

Betty and David Koetser Foundation for Brain Research

Forschungskredit of the University of Zurich

Title: Single motor unit recordings reveal vestibular projections to the splenius capitis neck muscles in humans

Authors: *S. M. ROSENGREN[1,3], K. P. WEBER[4], D. L. DENNIS[5], S. GOVENDER[5], M. S. WELGAMPOLA[3,2], J. G. COLEBATCH[5];
[1]Royal Prince Alfred Hosp., Camperdown, Australia; [2]Inst. of Clin. Neurosciences, Royal Prince Alfred Hosp., Sydney, Australia; [3]Central Clin. Sch., Univ. of Sydney, Sydney, Australia; [4]Dept. of Ophthalmology and Dept. of Neurol., Univ. Hosp. Zurich, Zurich, Switzerland; [5]Prince of Wales Clin. Sch. and Neurosci. Res. Australia, Univ. of New South Wales, Sydney, Australia

Abstract: The vestibulo-collic reflex (VCR) in humans is well-defined for only the sternocleidomastoid (SCM) neck muscle. However, other neck muscles also receive input from the balance organs and participate in neck stabilization. We therefore investigated the sound-evoked VCR projection to the splenius capitis (SC) muscles. We compared surface and single motor unit responses in the SCM and SC muscles in 10 normal volunteers. We stimulated with 2 ms tone bursts of 500 Hz in the left and right ears. The strongest responses were recorded in the SCM ipsilateral to the stimulated ear and the SC on the contralateral side. In both cases there was a significant decrease or gap in single motor unit activity: in SCM at a median latency of 13 ms for 36/41 units, and in SC at 14 ms for 40/61 motor units. In contrast, there were very few significant responses in the contralateral SCM and ipsilateral SC muscles, and they tended to be increases in activity. Surface responses in the ipsilateral SCM consistently showed a biphasic positive-negative wave with peaks at 13 and 23 ms, while there were occasional/smaller negative-positive responses on the contralateral side. Surface responses over the ipsilateral SC were inconsistent, while those over the contralateral SC were positive-negative during neck rotation and negative-positive during neck extension. An initial decrease in single motor unit activity suggests an inhibitory projection, while an increase indicates an excitatory projection. The results thus demonstrate an uncrossed inhibitory vestibular projection to the ipsilateral SCM and a crossed inhibitory projection to the contralateral SC. There was a weak excitatory projection in some subjects in the complementary muscle pair. This pattern of activity is consistent with the agonist relationship between these muscles. Surface responses over SC were unreliable indicators of reflex polarity, while needle recordings unambiguously showed the polarity of the reflex.

Disclosures: S.M. Rosengren: None. **K.P. Weber:** None. **D.L. Dennis:** None. **S. Govender:** None. **M.S. Welgampola:** None. **J.G. Colebatch:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.11/U2

Topic: D.07. Vestibular System

Support: PIOF-GA-2013-624158

Title: Our internal model of head and neck control incorporates electrical vestibular stimulation as a self-generated sensory signal

Authors: *P. A. FORBES[1,2], J. C. FICE[2], A. C. SCHOUTEN[1,4], G. P. SIEGMUND[5,3], J.-S. BLOUIN[2];
[1]Delft Univ. of Technol., Delft, Netherlands; [2]Sch. of Kinesiology, [3]Univ. of British Columbia, Vancouver, BC, Canada; [4]Univ. of Twente, Twente, Netherlands; [5]MEA Forensic Engineers & Scientists, Richmond, BC, Canada

Abstract: Differentiating sensory input from externally imposed movements (i.e., exafference) versus self-generated movements (i.e., reafference) is essential for orienting the head. Vestibular signals from self-generated movements are suppressed when actual sensory information matches expected sensory feedback using an internal model. Recent work suggests that this internal model is recalibrated (i.e. updated) to account for vestibular signals that are artificially modulated with the ongoing head motion. For example, in standing subjects, electrical vestibular stimulation (EVS) that is coupled to head angular velocity can be incorporated into the internal model after a conditioning period that includes reliable visual or somatosensory cues. Here, we directly tested this proposal during head-neck control by coupling EVS to head pitch velocity with the head constrained to move only in pitch. Our aim was to estimate how much of an exafferent head-coupled EVS can be transformed into a reafferent signal. Because EVS evokes a perceived head roll rotation (i.e., lateral bending), we expected lateral forces applied by the head to increase when the stimulus was applied and to return to normal after a conditioning period with eyes open (EO). Subjects voluntarily flexed and extended their head ±13° at 0.4 Hz while attached to a robot that constrained head roll and yaw. Head-neck forces and moments were measured while subjects moved continuously during 6 sequential trials: 1) baseline, no EVS with eyes closed (EC), 2) head-coupled EVS with EC, 3) head-coupled EVS with EO, 4) head-coupled EVS with EC, 5) no EVS with EC, and 6) no EVS with EO. During head-coupled EVS (Trial 2), lateral head-neck forces increased relative to no EVS with EC (Trial 1) and the force direction varied with the polarity of the EVS. Lateral forces quickly diminished when subjects opened their eyes and were given visual feedback of lateral forces (Trial 3). When subjects reclosed their eyes (Trial 4), lateral forces remained similar to equivalent no EVS conditions (Trial 1) despite receiving EVS. When EVS was then removed (Trial 5), lateral forces increased to levels similar to when EVS was first applied but in the opposite direction. These lateral forces were quickly diminished when subjects reopened their eyes (Trial 6) with visual feedback of their lateral forces. Our results show that for head-neck control, the central nervous system can recalibrate isolated exafferent vestibular only signals as reafferent signals by using reliable visual feedback of head motion and force. Moreover, this recalibration is complete, since when the external signal is removed, an equal and opposite effect occurs.

Disclosures: **P.A. Forbes:** None. **J.C. Fice:** None. **A.C. Schouten:** None. **G.P. Siegmund:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); MEA Forensic Engineers & Scientists. **J. Blouin:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.12/U3

Topic: D.07. Vestibular System

Support: R00 DC012775-03

Title: Developmental and circadian regulation of the locomotor contribution to balance in zebrafish

Authors: *D. E. EHRLICH[1], D. SCHOPPIK[2];
[1]Neurosci. and Physiol., NYU Langone Med. Ctr., New York, NY; [2]NYU Sch. of Med., New York, NY

Abstract: While locomotion subserves goal-directed behaviors it can also promote the maintenance or restoration of balance. Tight regulation of balance is required for survival and must be acquired early in life. As balance improves throughout development, we hypothesized locomotion more effectively regulates posture with age. To this end, we defined the contribution of swimming to balance in developing zebrafish larvae (age 5 to 28 days). We constructed an apparatus that records both the orientation of the long axis of freely swimming fish relative to the horizon and their position in the water column. Fish were monitored over 48 hour periods with a 14/10 light/dark cycle. Zebrafish larvae swim in short, discrete bouts that correct deviations in pitch from the preferred, horizontal orientation. During intervals between bouts larvae steadily rotate in pitch, accruing eccentricity with time. Propulsive bouts produce compensatory rotations that negatively correlate with initial pitch. In addition, shorter latencies to propulsion are observed for more eccentric pitches. Fish produce larger propulsive rotations and deviate less from horizontal pitch as they mature. Early but not late in development, larvae deviate more from horizontal pitch in the dark phase than the light phase. Immature larvae in the dark phase reach more eccentric pitches before propulsion, exhibit longer delays between propulsive bouts, and generate propulsive rotations twice the magnitude of those in the light phase. Together, these data suggest rotations produced during locomotion compensate for pitch eccentricity, thereby restoring balance. In addition, zebrafish posture varies and balance is maintained more effectively across age and the light/dark cycle. Future experiments will address the causal relationship between pitch eccentricity and locomotor compensation.

Disclosures: D.E. Ehrlich: None. **D. Schoppik:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.13/U4

Topic: D.07. Vestibular System

Support: R00 DC012775-03

Title: Swimming blindly: a role for vision in postural maintenance of larval zebrafish

Authors: *S. D. SUN[1], D. E. EHRLICH[2], D. SCHOPPIK[2];
[1]New York Univ., New York, NY; [2]NYU Sch. of Med., New York, NY

Abstract: Visual feedback is thought to modulate somatosensory and vestibular sensations, enabling both proper acquisition and maintenance of balance. This multisensory integration must happen on both short and long timescales to permit both rapid corrective movements and proper gait development. The precise mechanisms for such integration remain poorly understood. We have developed a novel behavioral paradigm using freely swimming zebrafish (Danio rerio) to explore how acute and chronic perturbations of visual input modulate posture. Of the three cardinal axes, previous work has characterized zebrafish movements in yaw and roll. Comparatively little is known about posture in pitch (nose-up/nose-down). We constructed an apparatus that records both the orientation of the long axis of the fish relative to the horizon and its position in the water column. We analyzed the static pitch, dynamic rotations, and translation of normal, dark-reared, and blind larval zebrafish swimming in both the light and dark. Blind zebrafish are most likely to be pitched level with the horizon, 0.4º±18º (mean±SD). In contrast, wild-type zebrafish have a slight nose-up bias to their posture 10.1º±14º. Dark-reared zebrafish are pitched similarly to blind zebrafish, near the horizon; however, their posture varies like that of wild-type zebrafish at 1.3º±13°. Zebrafish larvae swim in short discrete bouts. We observed a strong rotational component during both the inter-bout interval and the swim bout itself. On average, all fish rotated nose-down during the inter-bout interval, and appear to compensate during the bout with a nose-up rotation. Both blind and dark-reared larvae swam less frequently than sighted siblings. As such, there were larger nose-down deviations in posture before a corrective propulsive bout. During corrective propulsion, blind and dark-reared larvae exhibit higher peak angular velocities than sighted fish, compensating for these larger nose-down deviations. We infer that blind and dark-reared larvae detect nose-down changes later than normal larvae and as a result must correct more for such changes. Interestingly, though dark-reared zebrafish exhibit some postural characteristics similar to blind zebrafish, they appear to have longer propulsive bouts than both sighted and blind fish. Our data suggest that visual information is fundamental to maintain a normal pitch, and may help to guide compensatory movements for pitch changes during periods of inactivity. These results provide a foundation for

further investigation into the role of vision in the maintenance of balance and posture during larval zebrafish development.

Disclosures: **S.D. Sun:** None. **D.E. Ehrlich:** None. **D. Schoppik:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.14/U5

Topic: D.07. Vestibular System

Support: NIH R01 NS-058659

Barrow Neurological Foundation

Title: Feline head movement during walking

Authors: H. N. ZUBAIR[1], M. Y. IZADY[1], H. SUN[2], V. MARLINSKI[1], *I. N. BELOOZEROVA[1];
[1]Neurobio., [2]Neurosurg., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: How cats move their heads while walking has not been studied in detail so far. Given that cats are a popular and otherwise well-researched animal model for studies of posture and visual control of locomotion, this is an important shortage. The goal of this study was to investigate head movement in the cat during walking. Three cats walked in a chamber 2.5 meters long and 0.3 meters wide either under normal laboratory illumination or complete darkness. Position and orientation of the head was recorded with a motion capture and analysis system (Visualeyez, PTI, Canada) using three infrared LEDs placed on the head. 3D positions of cats' right shoulder and forelimb wrist were also recorded using LEDs. The sampling frequency of all recording was 200 Hz. Under the two lighting conditions, cats walked steadily through the test corridor with average speed of 0.59 ± 0.12 m/s (mean ± SD). The head accelerated in the fore-aft direction twice per step cycle: at the beginning and end of stance phase. The head oscillated along the vertical axis in a 12.0 ± 3 mm range twice during the cycle, with one peak in middle of swing and one in middle of stance of the right forelimb. Peaks of vertical acceleration with a range of ± 2 m/s^2 occurred at beginning and end of stance. The head oscillated in the medio-lateral direction in a 7.6 ± 2.2 mm range one time during the cycle, with maximal displacement to the left at beginning of stance of the right forelimb. Lateral acceleration to the left was highest in the middle of stance. Pitch of the head oscillated from -16 to -32 degrees and the range was 7.4 ± 1.6 degrees peak to peak. There were two oscillations for every step cycle: pitch was lowest in middle of swing and in middle of stance. Pitch velocity also oscillated twice per step cycle, and was highest near the end of swing and end of stance. Roll of the head on average ranged by 2.0 ± 0.4 degrees, and had one peak per step cycle: maximum roll to the left occurred

at beginning of stance of the right forelimb. Yaw of the head was 1.9 ± 0.9 degrees. Yaw rotations did not follow any obvious pattern between trials. Two of three cats had similar walking trends (velocity, stride length, step duration, stride duty factor) in the darkness and under illumination. These cats, in darkness, walked closer to the chamber's central wall by 10 mm and rotated their head toward it by 5 degrees. The other cat displaced its head downward 10 mm and also rotated it downward 7.5 degrees during walking in darkness. The data showed that, during walking on the flat surface, the head of the cat rhythmically displaces and rotates in all directions in synchrony with the step cycle. The differences between light and dark conditions were small.

Disclosures: H.N. Zubair: None. **M.Y. Izady:** None. **H. Sun:** None. **V. Marlinski:** None. **I.N. Beloozerova:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.15/U6

Topic: D.07. Vestibular System

Support: NIH Grant DC04158

Title: Evidence for sensory signal filtering for perceptual decision-making

Authors: *D. M. MERFELD, F. KARMALI;
Otol & Laryngol, Harvard Med. Schl, Boston, MA

Abstract: Perception can be attended in different ways. For example, when the vestibular system transduces self-motion, subjects can focus on different aspects of the perception. More specifically, during rotation, humans can focus on the amount of rotation perceived, or the perceived direction of rotation. Different perceptual features utilize different neural pathways that likely include signal processing appropriate to the perception of interest. For example, high-pass filter characteristics have been described for perception of rotational velocity (1), for perceptual thresholds during a direction recognition task demonstrate (2,3,4), as well as for semicircular canal afferent signaling (5). Some authors have suggested that each of these filters is distinct, while others have suggested that some may reflect the same underlying processing. To investigate this question, we measured both the magnitude of perceived angular yaw rotation and perceptual yaw rotation direction recognition thresholds in a population of 8 human subjects on a low-vibration rotator. Thresholds were measured by asking subjects to report whether they perceived a leftward or rightward rotation when rotated using cosine velocity profiles at different frequencies. As reported before, we found that thresholds increased as frequency decreased. As for earlier studies a high-pass filter time constant consistent with the data was determined using

generalized linear model fits. Magnitude of perceived rotation was measured by rotating the same subjects using 50 deg/s velocity steps and asking them to press a button every time they rotated through 90 deg. All subjects demonstrated an exponential decay in perceived angular velocity. We found the average time constant for magnitude estimation was 20 s; the average time constant for direction recognition was 1.2 s. These values are substantially and significantly different (paired t-test, p=0.0006). These results suggest the existence of a high-pass filter specifically for the decision-making process, which is distinct from other high-pass filtering for vestibular sensation and perception. (1) Bertolini et al. 2011, J Neurophysiol 105.1: 209-223. (2) Grabherr et al. 2008 Exper Brain Res 186.4: 677-681. (3) Soyka et al. 2011 Exp Brain Res 209.1: 95-107. (4) Coniglio et al. 2014 J Assoc Res Otolaryngol 15.2: 305-317. (5) Goldberg et al. 1971 J Neurophysiol 34:635-660.

Disclosures: D.M. Merfeld: None. **F. Karmali:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.16/U7

Topic: D.07. Vestibular System

Support: NSERC Discovery Grant LRB

Title: Can vision be used to recalibrate vestibular feedback for perturbation recovery?

Authors: *A. J. TOTH[1], L. R. HARRIS[2], L. R. BENT[1];
[1]Human Hlth. and Nutritional Sci., Univ. of Guelph, Guelph, ON, Canada; [2]Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: Background: The successful recovery of balance in response to a perturbation requires the appropriate integration of somatosensory, visual, and vestibular feedback. Through the use of galvanic vestibular stimulation (GVS), previous work has shown that vestibular information is used during the later stages of perturbation recovery as the body realigns to a perceived representation of vertical [1]. We also know that the vestibular signal for verticality can be recalibrated using visual feedback as shown during gait [2]. The current study asks whether vestibular input is also calibrated to a visual vertical for perturbation recovery.
Methods: Subjects stood on a motion platform and received binaural bipolar GVS at a level 2X their vestibular threshold (threshold: lowest current able to evoke head movement). Subjects wore PLATO visual occlusion spectacles (Translucent Technologies Inc.)**,** used to control the timing and availability of visual information. Kinetic and kinematic data were collected to estimate centre of pressure (CoP) and centre of mass (CoM) movements. During test conditions, subjects received GVS for 4 seconds in the absence of vision. With ongoing GVS, subjects

regained vision for 4 seconds and realigned their altered body position to their visual surround. Vision was then re-occluded and GVS turned off 500ms prior to an antero-posterior (A-P) platform perturbation. GVS and perturbations in control conditions were identical to those used in the test condition. However, subjects were not provided a period of visual availability with which to aid postural realignment. **Preliminary results** indicate that both the peak ML CoM and CoP late responses to the A-P perturbation in the test condition, following the end of the GVS signal, had significantly greater alterations of final equilibrium position relative to vertical compared to controls (CoM; $p = 0.045$, CoP; $p = 0.046$). These findings suggest that a prior period of visual feedback can affect how vestibular information is used to realign posture during perturbation recovery. By using vision to facilitate postural realignment in the presence of GVS, we believe subjects are recalibrating vestibular input to align with the visual vertical. After this recalibration, turning off GVS during perturbation recovery will, at least temporarily, alter the final equilibrium position to the newly 'set' visual/vestibular vertical. The implications of this work align with the consequences of recalibrating vestibular input to erroneous visual signals; these may have profound effects on balance control in elderly populations. [1] Inglis T, et al. (1995) J Neurophysiol. [2] Sturnieks D, et al. (2005) Gait and Pos

Disclosures: **A.J. Toth:** None. **L.R. Harris:** None. **L.R. Bent:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.17/U8

Topic: D.07. Vestibular System

Title: Human spatial orientation is distinctly task-dependent

Authors: ***N. BURY**, O. BOCK;
Inst. for Physiol. and Anat., German Sport Univ. Cologne, Cologne, Germany

Abstract: Spatial orientation in humans is based on three reference frames - gravicentric (pull of Gravity), allocentric (alignment of familiar visual objects) and egocentric (orientation on the own body). We have shown before that in absence of allocentric information, participants rely mainly on egocentric rather than on gravicentric cues for control of their hand movements. Here we evaluate whether egocentric cues prevail for other response types as well. Twenty-four participants (12 male, 12 female; 22.1 ± 2.9 years) were placed in-to three angles of pitch (upright 0°, supine -90°, head-down tilt -110°) inside a gym wheel. They were blindfolded to eliminate allocentric cues. In test T1, their task was to adjust the pitch angle of a schematic tree drawing until "⋯the leaves are at the top and roots are at the bottom"; in test T2, their task was to stop their passively moved forearm when it intuitively appeared horizontal or vertical; in test T3, their task was to verbally indicate their own body orientation with respect to the thought face

of a clock. At -90° and -110° pitch, responses in T1 were bimodally distributed such that one mode coincided with the gravicentric and the other mode with the egocentric reference frame. In contrast, responses in T2 and T3 were unimodal, coinciding with only the gravicentric reference frame. We conclude that different perceptual judgements utilize gravicentric and egocentric cues differently, and motor responses utilize them yet differently. In consequence, there seems to exist more than one single internal representation of space.

Disclosures: **N. Bury:** None. **O. Bock:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.18/U9

Topic: D.07. Vestibular System

Title: The influence that modulation of subjective postural vertical decrease awareness of body orientation

Authors: *T. SHIOZAKI, Y. OKADA, S. MORIOKA;
Kio Univ., Nara-Ken, Japan

Abstract: Background Subjective postural vertical (SPV) is a method used to assess perception of verticality. It is reported that in patients who exhibit contraversive pushing, the bias of SPV is larger than in other stroke patients, and as the patients improved with respect to pushing, the bias of SPV disappeared. Use of visual information has been used as a treatment strategy for pushing and it is proposed that gaining vertical position repeatedly and being consciously aware of their verticality produces favorable results. Therefore, we investigated whether the bias in the perception of body verticality influenced decreasing awareness of body orientation, and whether it can be regained with visual information. Methods Subjects were young healthy volunteers. A rotating chair was used for assessing the SPV tilt by 1.5 deg per second on the frontal plane. The SPV corresponding to the angle of body tilt relative to the direction of earth's gravity was measured with a spatial accuracy of 0.1deg. To induce a bias in the SPV experimentally, and in order to assess whether the participants were objectively tilted but subjectively upright, participants were laterally tilted for 5 min at 20 deg. They were then slowly brought back toward earth's vertical, and were asked to press the switch when they had reached a subjective upright position. In the condition in which participants were subjectively and objectively tilted, the participants were brought back to the position of the biased SPV measured in the previous condition. We assumed that the subjects perceived the tilt of their own bodies when a picture of a rich landscape was shown to indicate verticality. We tested whether the reaction time differed in the two conditions. SPV was measured under the conditions when a landscape picture was shown, and it was investigated whether tilt of a body could be recognized accurately by the

visual information. Results The SPV bias was 3.21±2.31°from the earth's vertical under normal conditions. The SPV after lateral body tilt (5.01 ± 2.82°) was statistically different from normal ($P < 0.05$). The participants who were objectively tilted but subjectively upright showed a delay in reaction time and judged their own direction of tilt in comparison with subjective and objective tilt. The angle was 1.34 ± 1.14° and the SPV bias in which visual information was used was small compared with the normal and after the lateral body tilt. Conclusion These data suggest that inducing SPV bias for lateral body tilt decreases awareness of body orientation. Futures studies will need to investigate the mechanism of improvement of regaining awareness of body orientation by using of visual information.

Disclosures: **T. Shiozaki:** None. **Y. Okada:** None. **S. Morioka:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.19/U10

Topic: D.07. Vestibular System

Support: NIH Grant K23DC013552

Leon Levy Foundation

Title: Drift of torsional eye movements and perception of upright during prolonged head tilts

Authors: *J. OTERO-MILLAN[1], A. KHERADMAND[2];
[1]JOHNS HOPKINS UNVIVERSITY, Baltimore, MD; [2]Neurol., Johns Hopkins Unversity, Baltimore, MD

Abstract: Torsional eye movements are rotations of the eye around the line of sight and they occur when we tilt our head towards the shoulder. Perception of upright is usually assessed by a psychophysical task known as subjective visual vertical (SVV) which quantifies the orientation of a visual line that a subject perceives as aligned with the direction of gravity. Torsional eye movements change the orientation of the visual stimulus on the retina thus playing a critical role in our perception of upright. Previous studies have shown that both torsion and SVV can drift over time during prolonged head tilts. Here we directly compared the amount of drift of torsion and drift of SVV during continuous recordings of 15 minutes. We used a novel video tracking method to measure torsion and an adaptive paradigm to continuously measure SVV. We found that torsion always drifted towards zero (the eye position during upright) by an average of 1.5 degrees and between 0 and 3 degrees depending on the subject. SVV on the other hand was much more variable across subjects with a drift ranging from 0 to 10 degrees and an average of 4 degrees towards the direction of the head tilt. The amount of drift in torsion and SVV was not correlated across subjects. That is, subjects with larger torsional drifts did not necessarily have

larger SVV drifts. The mechanisms for the drifts of torsion and SVV remain unknown but our results suggest that they are independent. The precision of SVV measured with the psychometric curve remained constant in spite of the drifts of the accuracy of SVV suggesting that fatigue or lack of attention are not the sources of the drift.

Disclosures: J. Otero-Millan: None. **A. Kheradmand:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.20/U11

Topic: D.07. Vestibular System

Support: Pervasive and Ambient Computing Lab, Loyola University Chicago

Assistantship Biology Dept. Loyola University Chicago Graduate School

Title: Bayesian analysis of perceived eye level

Authors: *E. E. ORENDORFF[1], R. T. PALUMBO[2], M. V. ALBERT[3];
[1]Biol., [2]Psychology, [3]Computer Sci., Loyola Univ. Chicago, Chicago, IL

Abstract: Perceived eye level (PEL) is a self-reported measure of elevation angle based on visual and internal (proprioceptive/vestibular) cues. It is a valuable reference point when judging height and distance. We applied a Bayesian framework to estimate the underlying computations performed by subjects in the determination of PEL from sensory inputs. The model assumes normal distributions of PEL from individual cues combine to produce a single normal distribution. We calculated separate variance estimates for each input, using the experimental data available - where internal cues were controlled and visual cues consisted of short-12° or long-64° pitched-from-vertical lines, with pitches ranging from -30 to 30°. In total darkness, relying on internal input alone, PEL was near true eye level with SD = ±1°. When subjects were presented with one short line stimulus, visual input had greater variance (SD = ±2.46°), than when subjects were presented with one long line stimulus (SD = ±1.45°). Using these variance estimates to predict the effect of two short or two long line stimuli on PEL, the predicted means were close to those observed (SEE = ±0.73 and ±3.52, respectively). Our model provides a parsimonious explanation for the additive effect of low fidelity cues and the averaging effect of high fidelity cues, as found in other Bayesian cue combination psychophysical studies. The model accurately predicts perceived eye level and provides a means of deriving an appropriate analytical function. We demonstrate this model in a re-interpretation of results by Matin and Li (2001). Vision Res. 41:2845-2872.

Disclosures: E.E. Orendorff: None. **R.T. Palumbo:** None. **M.V. Albert:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.21/U12

Topic: D.07. Vestibular System

Title: Aging and the vestibular system

Authors: *C. DE WAELE[1,2], E. CHIAROVANO[3,4], G. LAMAS[2], P.-P. VIDAL[3];
[1]Cognac G, CNRS UMR 8257, Paris, France; [2]Pitie Salpetriere Hosp., Paris, France; [3]Cognac G, Paris, France; [4]Univ. Paris Descartes, Paris, France

Abstract: Objectives: To investigate the vestibular receptors function and the static equilibrium in seniors. We tested the hypothesis that vestibular dysfunction may vary in function of the type otolithic or canalar tested. Methods: Data were obtained in 166 patients over 65 years of age in the ENT department of the Pitie-Salpetriere hospital. Vestibular function was assessed using caloric, vHIT test and ocular and cervical VEMPs. VHIT consisted of passive and unpredictable, head rotation in the vertical (LARP and RALP) and in the horizontal planes (lateral). Cervical and ocular vestibular evoked myogenic potentials were recorded in response to air conductive or bone conductive stimulation (BK 4810). The Sensory Organisation Test (EquiTest) was used to quantify the role of vestibular, visual and proprioceptive inputs in maintaining balance. The velocity of the displacement of the center of pressure of the feet was measured on the wBB and on the wBB plus foam in eyes open and eyes closed conditions. Finally, patients were asked to fill in the Dizziness Handicap Inventory (DHI) questionnaire. Their cognitive function was analyzed using CANTAB. The aim was to try to detect the risk of falls. Results: We failed to observe a dysfunction of the horizontal and anterior canal receptors using calorics and vHIT in senior. In contrast, vHIT data in the vertical plane showed lower gain when the posterior canal was tested compared to healthy young subjects. We also showed a decreased in the excitability of the utriculo-ocular (n1-p1 waves, 52%) and sacculo-spinal pathway (P13-N23 waves, 37%). Abnormal Equitest (falls in condition 5 or 6) was more often observed in patients with low scores to DHI. Finally, Equilibrium was normal on foam in eyes open conditions. However, 20% of the seniors fell when tested in the foam in eyes closed condition. The velocity of XY pathway of the COG on the foam eyes closed was 4.5±1.0 cm/sec for seniors 80-89 aged and 2.65±0.5 cm/sec for young 30-39 aged. Conclusion: This study showed an alteration of the function of some vestibular receptors with age: utricle, saccule and posterior canal. In addition, the performances of standing upright on foam in eyes closed condition decreased. These results suggested that falls in seniors may be partly related to dysfunction of otolith and posterior vestibular. In eyes closed, vestibular inputs provide absolute information about the body's orientation explaining why standing upright became more difficult and increased mental load.

Disclosures: **C. de Waele:** None. **E. Chiarovano:** None. **G. Lamas:** None. **P. Vidal:** None.

Poster

335. Vestibular Perception, Posture, and Spatial Orientation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 335.22/U13

Topic: D.07. Vestibular System

Support: NHMRC 1027089

Title: Sensory and reflexive hypersensitivity in mal de debarquement syndrome

Authors: *R. C. FITZPATRICK, S. R. D. WATSON;
Univ. of New South Wales, Sydney, Australia

Abstract: *Mal de Debarquement* (MdD) is a persistent imbalance syndrome classically after a sea voyage. It presents as abnormal non-vertiginous motion sensations, typically rocking and bobbing. There is no consensus on pathogenesis and aetiology other than it is probably not primary vestibular as clinical vestibular function tests are normal and it is unresponsive to vestibular treatment. We aimed to determine if MdD patients are hypersensitive to vestibular signals of rotation with the hypothesis that they would show perceptual but not reflex hypersensitivity. Patients (N=8, 7F, 27-60yrs) and matched controls were studied. All had normal vestibulo-ocular head impulse and caloric tests. *Perceptual sensitivity* to whole-body yaw and lateral rocking were determined by psychophysical tests. In dark, seated subjects were rotated 30-180° over 5s before pointing to their start positions. Illusory motion evoked by galvanic vestibular stimulation (GVS: 1.5mA, 10s) was used to assess pure vestibular perception. *Sensory thresholds* for detecting the direction of yaw were determined by forced-choice tracking and fitting a psychometric function to identify the amplitude that produced 50% correct responses. Rocking sensitivity was determined sitting on a motorised swing with a roll axis aligned with the head. Subjects reported perceptions of rocking (1-4°, 0.3Hz) using a visual scale. Rocking sensitivity was similarly determined using sinusoidal GVS (0.25-4mA) while stationary. Balance during standing was assessed by forceplate posturography with eyes open and shut, and standing on a rigid floor and foam. *Vestibulospinal reflexes* were assessed by binaural bipolar GVS (≤1mA) measuring medium-latency lateral shear forces. Posturographic measures from MdD patients were not different to those of control subjects. MdD patients showed exaggerated senses of self-motion during real yaw ($P=0.02$ by ANOVA) or virtual yaw ($P<0.001$). However, MdD thresholds for detecting yaw direction were increased ($P=0.007$). MdD patients showed exaggerated senses of roll self-motion during real rocking ($P=0.02$) and virtual rocking ($P<0.001$). Against our hypothesis, MdD showed markedly increased and delayed medium-latency GVS responses during standing ($P<0.001$). *Conclusions.* MdD is characterised by perceptual motion hypersensitivity and vestibulospinal hyper-reflexia, but normal VOR responses. The results indicate separation of perceptual, balance and ocular processing of

vestibular afference. MdD could reflect disordered autoregulation of vestibular sensitivity or integration with somatosensory and visual afference as occurs in normal subjects.

Disclosures: R.C. Fitzpatrick: None. **S.R.D. Watson:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.01/U14

Topic: D.08. Pain

Support: NIH 1R01DE022129-01A1

Title: Local field potential changes in the lateral thalamic nuclei of behaving rats using modified Gi protein coupled receptor

Authors: *J. N. STRAND[1], C. STINSON[2], Y. B. PENG[1], L. L. BELLINGER[2], P. KRAMER[2]; [1]Univ. of Texas At Arlington, Arlington, TX; [2]Dept. of Biomed. Sci., Baylor Col. of Dentistry, Texas A&M Univ., Dallas, TX

Abstract: The present study analyzes the summated electrical activity of the lateral thalamic nuclei compared to pain behaviors in a trigeminal model of pain in freely behaving rats. The lateral thalamus processes somatosensory information from the head and facial muscles and is therefore pivotal in the perception of pain from nociceptive input at those sites. Changes in the neuronal activity of the lateral thalamus should influence changes in pain perception as seen by pain behaviors. To affect changes in neuronal activity, a recently developed approach of DREADD (Designer Receptors Exclusively Activated by Designer Drugs) is used to activate a modified G protein coupled receptor (mGPCR) in the targeted region. Activation of the mGPCR triggers the inhibitory Gi protein; Gi is expected to facilitate neuronal silencing. Using local field potential to record the thalamic neuronal activity changes due to mGPCR in freely moving animals provided a novel viewpoint of monitoring lateral thalamic neural activity during active pain. In these studies the lateral thalamus of Sprague-Dawley rats were injected with an adeno-associated virus vector (AAV8) containing a construct that expresses a modified acetylcholine receptor that had been modified to bind to clozapine-N-oxide (CNO) instead of its native ligand, acetylcholine. CNO activation of the virus induces activation of the mGPCR, changing the neuronal activity level in the targeted lateral thalamic region. 30 minutes after CNO injection (1 mg/kg), 50µL of 3% formalin was injected into the masseter tendon, inducing nociception. LFP and behavioral responses were recorded simultaneously in both drug and control groups. We found that the pain behaviors are significantly reduced in the drug group compared to the no drug group. We have further found that summated electrical activity is increased in the lateral thalamic region for the drug group, especially in the beta and gamma frequency bands. The

results from this study suggest that LFP can be a useful tool to monitor pain behavior in freely behaving animals.

Disclosures: **J.N. Strand:** None. **C. Stinson:** None. **Y.B. Peng:** None. **L.L. Bellinger:** None. **P. Kramer:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.02/U15

Topic: D.08. Pain

Support: MINECO SAF2014-54518-C3-1-R

MINECO SAF2014-54518-C3-2-R

CONACYT-203564

Title: Electrophysiological characterization of trigeminal neurons innervating the anterior structures of the eye

Authors: ***B. SANTIAGO**, M. C. ACOSTA, J. GALLAR, C. BELMONTE;
Inst. De Neurociencias UMH-CSIC, San Juan De Alicante, Spain

Abstract: The functional properties of the trigeminal ganglion neurons (TGN) innervating the cornea, conjunctiva, and eyelids and mediating sensations of pain and discomfort are incompletely known. The aim of this work was to characterize *in vivo* the firing characteristics of the different sensory types of TGN innervating the anterior structures of the eye. Anesthetized Wistar male rats (350±25g) were placed in a stereotaxic frame and units from the right TG were recorded extracellularly with tungsten electrodes (2-5MΩ). The signal was amplified, filtered and recorded using an ADC interface and software for off-line analysis. TGN were identified by stimulation eye tissues with a fine brush or approaching a cooled metal probe. The receptive field (RF) and the mechanical threshold (MT) were determined using von Frey filaments. Thermal (saline drops at 21ºC or at 50ºC) and chemical (98% CO2 gas jet; 100µM menthol; 850mOsm hyperosmolar saline) stimuli were applied onto the RF. Electrical pulses (Ag/AgCl bipolar electrode, 0.1-2ms, 5-30V) were applied to measure conduction velocity (CV). TGN innervating the bulbar (n=15) and tarsal (n=21) conjunctiva; the upper (n=15) and lower (n=15) eye lids, and the cornea (n=38) were analyzed. TGN were classified depending on their responses to the stimuli as polymodal, mechanical and cold TGN. All these types were found in the conjunctiva and cornea, while only mechanoreceptors were found in the eyelids, some associated to the eyelashes. A part of the polymodal TGN showed spontaneous activity that increased markedly with CO2 stimulation and mechanical pressure; MT was similar in all territories (0.14±0.06mN,

0.17±0.05mN, 0.21±0.04mN; cornea, bulbar and tarsal conjunctiva respectively). Mechanoreceptor neurons had no spontaneous activity and MTs that varied among tissues (0.08mN, 0.6±0.37mN, 0.19±0.05mN, 0.51±0.16, mN; eyelids, cornea, bulbar and tarsal conjunctiva respectively). Cold TGN fired rhythmically, increasing their frequency of discharge when cooling and silencing with heating. Two subpopulations of cold thermoreceptor neurons (low and high threshold TGN) were found in the cornea, distinguished by their spontaneous activity (8.01±0.81 imp/s, n=13 vs. 1.83±0.42 imp/s, n=10), cold threshold (-0.11±0.01ºC vs. -0.61±0.37ºC) and peak frequency (41±4.64imp/s vs. 17.2±3.56 imp/s). All TGNs explored showed CVs in the range of C (<2m/s) and Aδ fibers (>2m/s), regardless of the type and ocular region of origin. Finally, differences in stereotaxic coordinates showed the corneal and bulbar conjunctiva units more posterior and deep than tarsal units.

Disclosures: **B. Santiago:** None. **M.C. Acosta:** None. **J. Gallar:** None. **C. Belmonte:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.03/U16

Topic: D.08. Pain

Support: Japanese association for Oral Biology Grant-in-Aid for Young Scientists

Research Grant KAKENHI Grant-in-Aid for Young Scientist (B) #15K21410

Title: Satellite glial cell activation via extracellular signal-regulated kinase phosphorylation, associated with phenotypic change in trigeminal ganglion neurons, is involved in lingual neuropathic pain

Authors: ***A. KATAGIRI**[1], H. SAITO[4], K. OHARA[2], M. SHINODA[3], A. TOYOFUKU[5], K. IWATA[3];
[2]Dept. of Endodontics, [3]Dept. of Physiol., [1]Nihon Univ., Chiyoda-Ku Tokyo, Japan; [4]Dept. of Prosthodontics, Nihon Univ. Sch. of Dent., Chiyoda-ku Tokyo, Japan; [5]Dept. of Psychosomatic Dent., Tokyo Med. and Dent. Univ. Grad. Sch., Yushima Bunkyo-ku Tokyo, Japan

Abstract: Satellite glial cell (SGC) activation and associated phosphorylation of extracellular signal regulated kinase (ERK) in the trigeminal ganglion (TG) are known to be involved in trigeminal neuropathic pain associated with trigeminal nerve injury. However, the involvement of these molecules in orofacial neuropathic pain mechanisms is still unknown. Phosphorylation of ERK in lingual nerve crush (LNC) rats was observed in SGCs. In order to evaluate the role of neuron-SGC interactions in tongue neuropathic pain, calcitonin gene related peptide (CGRP)-immunoreactive (IR) neurons, phosphorylated ERK (pERK)-IR SGCs and glial fibrillary acidic protein (GFAP)-IR SGCs in the TG were studied in LNC rats. The number of CGRP-IR TG

neurons and TG neurons encircled with pERK-IR SGCs or GFAP-IR SGCs was significantly larger at day 3 after LNC than for sham or naïve rats. Percentage of medium and large sized CGRP-IR TG neurons was higher in LNC rats compared with sham or naïve rats. Following CGRP receptor blocker CGRP8-37 or mitogen-activated protein kinase/ERK kinase 1 inhibitor PD98059 administration into the TG for 3 days after LNC, the number of CGRP-IR neurons and neurons encircled with pERK-IR SGCs or GFAP-IR SGCs, activated SGCs, was decreased. The decreased nociceptive thresholds to mechanical and heat stimulation to the tongue were also significantly recovered. The present findings suggest that CGRP released from TG neurons activates SGCs through ERK phosphorylation resulting in the enhancement of TG neuronal excitability. The phenotypic switching of large myelinated afferent TG neurons expressing CGRP may account for neuropathic pain behavior.

Disclosures: A. Katagiri: None. **H. Saito:** None. **K. Ohara:** None. **M. Shinoda:** None. **A. Toyofuku:** None. **K. Iwata:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.04/U17

Topic: D.08. Pain

Title: Mental nerve constriction alters the motivation for self-administration of a sucrose solution, study in rats

Authors: *N. E. GUTIÉRREZ[1], D. QUIÑONEZ[2], A. GARCÍA URBINA[2], R. MORALES[2], I. O. PEREZ MARTINEZ[2];
[1]Univ. Nacional Autónoma De Mexico, Cuautla, Mexico; [2]Fes Iztacala, Univ. Nacional Autónoma De Mexico, Mexico, Mexico

Abstract: Chronic pain causes depression, anxiety and cognitive deficits that have as common feature a decrease in the motivation to complete goal-directed actions, and reductions in pre-pain activities, motivation is regulated by structures like hypothalamus and nucleus accumbens.The aim of this research is to evaluate how motivation of sucrose consumption changes during orofacial chronic pain caused as a result of trigeminal nerve injury.This study develops a method to measure motivation for sucrose(10%) consumption in a self-administration schedule and the modifications after orofacial chronic pain generated by mental nerve constriction.To measure motivation we used male Wistar rats (n=6 by group),weight 250 ±25 gr., food ad libitum,they were water deprived for 23 hrs. previous each session of operant self-administration, they had to press a lever to obtain a reward in a fixed ratio(FR) schedule, sessions were 40 minutes long and reward was water, when rats learnt the behavior, water privation was removed and rats had food and water ad libitum, rats continue on a fixed ratio schedule(1:1),but now reward was sucrose

(10%).Rats were two weeks in FR1,two days in FR2,two days in FR3, two days in progressive ratio(PR) schedule in which it becomes progressively more difficult to earn each subsequent reward, the first day they were in PR with step size 3, and the second the step size was 5.We divided animals in two groups, mental nerve constriction group and sham group.After the surgery they were with the same protocol of operant self-administration.The point at which the rats give up, provides a measure of motivation to work for reward.Finally, we used the two-bottle preference test to evaluate the effect of trigeminal nerve injury on sucrose preference behavior.We found that the latency time for the first response during FR1 was shorter in nerve injury group(ANOVA, p=0.007)compared with sham group.There is no significant differences in the number of rewards neither number of lever responses.During the PR, we found differences higher significant between the sham group and the nerve injury group both for rewards(t test, p=0.005) as for responses(t test, p=0.006), showing a significant decreasing in the motivation for sucrose produced by mental nerve injury.In the two-bottle preference test results show that the preference for 10% of sucrose is higher than water in both groups, but comparing sham group versus lesion group the preference for sucrose is lesser(t test, p=0.04)but not for water intake(t test, p=0.32).We have a very clear results that mental nerve injury decreases motivation for intake sucrose, therefore we can use this behavioral analysis as measure of orofacial pain.

Disclosures: **N.E. Gutiérrez:** None. **D. Quiñonez:** None. **A. García Urbina:** None. **R. Morales:** None. **I.O. Perez Martinez:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.05/U18

Topic: D.08. Pain

Support: JSPS 23390461

Title: Imaging of the excitatory transmission in the trigeminal subnucleus caudalis within the sliced medulla oblongatae of mice

Authors: ***M. HIRAHARA**, N. FUJIWARA, K. SEO;
Niigata Univ., Niigata, Japan

Abstract: Introduction: In order to characterize the signal transmission in the trigeminal subnucleus caudalis (Vc), where the functional structure of local neuronal circuit has not been well understood, we applied a calcium imaging technique to observe excitatory signal propagation using slices of mice medulla oblongatae. We have succeeded in optically recording calcium images of the Vc in response to electrical stimulation at the entry zone of the trigeminal nerve root by creating new oblique sectioned slices. Materials and methods: The medulla

oblongatae were removed from 6-7 week-old male mice (C57/BL6J), weighing 18-25g, under deep anesthesia following an intraperitoneal injection of 4% chloral hydrate (480 mg/kg). These medullae were sliced in thicknesses of 600 μm in an oblique plane which contained the nerve root and the Vc in ice-cooled Krebs solution. Next, the slices were incubated with Rhod-2-AM for 90 minutes in order to load the fluorescence calcium indicator. After the loading of the indicator, each slice was perfused with the Krebs solution in the measurement chamber at 31 degrees. A bundle of fibers in the vicinity of the nerve root was stimulated with a single electrical pulse (current: 100μA, duration: 200μsecs). Successive fluorescence images of the stimulated slices were recorded every 1.2 msecs and the pixels that had increased were extracted to produce the calcium images. Results: The stimulation to the bundle of fibers around the trigeminal nerve root evoked an increase in intracellular calcium concentration ([Ca2+]in) in the Vc within several msecs. This elevated [Ca2+]in gradually decreased but partially remained for at least 300 msecs. Notably, the evoked elevation of [Ca2+]in was inhibited in the presence of CNQX, and the response of [Ca2+]in partially recovered after being washed out for 15 minutes. Conclusion: The evoked elevation of [Ca2+]in was observed in the newly created slice preparations and retained the communication between the trigeminal nerve root and the Vc. Our findings suggest that these calcium responses may be induced mainly in post synaptic neurons by excitatory transmission via glutamate receptors from trigeminal primary afferent inputs.
Supported by JSPS 23390461

Disclosures: **M. Hirahara:** None. **N. Fujiwara:** None. **K. Seo:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.06/U19

Topic: D.08. Pain

Support: KAKENHI 26861799

Title: Temporal alterations of morphological and physiological features in experimental tooth movement model rats

Authors: ***N. HASEGAWA**[1], A. SASAKI[1], K. TAKAHASHI[2], G. YU[2], N. SUDA[1], H. SAKAGAMI[2], K. ADACHI[2];
[1]Orthodontology, [2]Pharmacol., Meikai Univ. Sch. of Dent., Sakado, Japan

Abstract: There are animal models to investigate effects of orthodontic treatment, and many of them focused on morphological alterations. Although some investigations evaluated behavioral alterations, which induced by orthodontic force (e.g., intensity of face-grooming and head-withdrawal reflex excitability), establishment of objective experimental methods those allow us

to evaluate orthodontic force-induced pain quantitatively is still required. Recently, we have reported that alteration of jaw-opening reflex (JOR) excitability is able to be the one. In this model, continuous orthodontic force was applied by Ti-Ni coil spring to only right maxillary first molar and electrical current (200 μs) was applied to bilateral maxillary first molar at one (D1), three (D3) and seven (D7) days after the placement of coil spring to compare physiological features of JOR (e.g., threshold, latency and AUC) between left and right sides. In brief, orthodontic force-induced pain increased excitability of nociceptive reflex in right, but not in left, side, and excitation was observed as significant reduction of threshold for inducing JOR at D1 (55.4 ± 7.8 % vs left). The reduction of threshold was returned to the intact level at D3 and this was seen up to D7. Loading of continuous orthodontic force induced medial movement of the first molar, which was increased with progression of postoperative days (D1: 0.12 ± 0.14 mm, D3: 0.19 ± 0.05 mm, D7: 0.42 ± 0.02 mm). Interestingly, moving distance of the first molar was negatively related with JOR threshold alteration, which is also seen in the orthodontically-treated patients. To investigate the role of acid secretion from mature osteoclasts in JOR excitability alteration, the number of tartrate-resistant acid phosphatase positive osteoclasts was counted (D1: 3.5 ± 0.5, D3: 11.5 ± 0.5, D7: 31.5 ± 0.5) and negative relationship between the number of mature osteoclasts and JOR excitability alteration was suggested. Since the activation of satellite glial cells (SGCs) in the trigeminal ganglion (TRG) was induced by the trigeminal inflammation, glial fibrillary acid protein (GFAP) immunoreactive SGCs in TRG were investigated and activated SGCs were observed in the bilateral TRG. At D1, treatment with aspirin (100 mg/kg, 3 times/day) immediately after the placement of coil spring significantly increased right side threshold compared with left side. Taken together, 1) unilateral orthodontic force application increased excitability of bilateral sensory processing and 2) tonic inhibition of cyclooxygenase reduced the excitability at only inflamed region were suggested.

Disclosures: **N. Hasegawa:** None. **A. Sasaki:** None. **K. Takahashi:** None. **G. Yu:** None. **N. Suda:** None. **H. Sakagami:** None. **K. Adachi:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.07/U20

Topic: D.08. Pain

Support: NIH Grant DE023846

Title: TRPV1 and TRPA1 contribute to mechanical hyperalgesia and spontaneous pain in craniofacial muscle inflammation

Authors: S. WANG, Y. ZHANG, J. RO, *M.-K. CHUNG;
Neural & Pain Sci., Univ. Maryland Dent. Sch., Baltimore, MD

Abstract: The most prevalent symptoms in temporomandibular joint disorders (TMD) patients are pain in response to pressure on the muscles and spontaneous pain. However, the mechanisms underlying pressure-evoked pain or spontaneous pain in craniofacial muscles are not fully understood. TRPV1 is a receptor of capsaicin, which is also activated by noxious heat and acid. In addition to its role in thermal hyperalgesia in skin, TRPV1 mediates glutamate-induced mechanical hyperalgesia in craniofacial muscles. TRPA1 is activated not only by natural compounds, such as mustard oil, but also by multiple endogenous electrophilic substances. Since TRPA1 is implicated in cutaneous and muscle mechanical hyperalgesia in the spinal system, it is possible that TRPA1 is also involved in mechanical hyperalgesia of craniofacial muscles. The objective of this study was to determine whether TRPV1 and TRPA1 are involved in mechanical hyperalgesia and spontaneous pain following inflammation of the masseter muscle. Injection of complete Freund's adjuvant (CFA) into rat masseter muscle induced mechanical hyperalgesia that lasted more than two weeks. The mechanical hyperalgesia was partially and reversibly inhibited by TRPV1 antagonist, AMG9810, or TRPA1 antagonist, AP-18, suggesting TRPV1 and TRPA1 are involved in mechanical hyperalgesia under muscle inflammation. To evaluate spontaneous pain behaviors, we performed two assays. First, we evaluated changes in the facial grimace scale. Second, we counted a characteristic face wiping responses over the ipsilateral cheek. In both rats and mice, masseter injection of CFA showed significantly greater facial grimace scales than the vehicle injected group, which was maintained up to 3 days following CFA injection. The face wiping responese were also significantly greater in CFA- than the vehicle-injected group. When AMG9810 or AP-18 was injected into masseter muscle 1 day after CFA treatment, both facial grimace scale and face wiping responses were significantly attenuated. Such analgesic effects of the antagonists disappeared after 24 hours. Furthermore, when TRPV1-expressing afferents were ablated by preemptive intramuscular injection of capsaicin in mice, CFA-induced changes in facial grimace scale and face wiping responses were significantly less than those observed from the group pre-injected with the vehicle. These results suggest that TRPV1 and TRPA1 are not only involved in mechanical hyperalgesia but also in spontaneous pain under craniofacial muscle inflammation and that TRPA1 and TRPV1 molecules as well as the nociceptive afferents expressing these two channels could be salient targets for treating TMD.

Disclosures: **S. Wang:** None. **Y. Zhang:** None. **J. Ro:** None. **M. Chung:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.08/U21

Topic: D.08. Pain

Support: DE021804

DE024220

Title: Neuropathic pain in a mouse model of chronic constriction nerve injury does not correlate with changes in chloride reversal potential in trigeminal nucleus caudalis neurons

Authors: *A. M. CASTRO[1], W. GUO[2], C. RAVER[1], F. WEI[2], R. DUBNER[2], A. KELLER[1]; [1]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Neural and Pain Sci., Univ. of Maryland Dent. Sch. & Program in Neurosci., Baltimore, MD

Abstract: Changes in chloride reversal potential in rat spinal cord neurons have previously been associated with persistent pain in nerve injury and inflammation models. These changes correlate with a decrease in the expression of the potassium-chloride transporter, KCC2, and with increases in neuronal excitability. Here, we test the hypothesis that similar changes occur in mice with neuropathic pain induced by chronic constriction injury of the trigeminal infraorbital nerve (CCI-ION). Our mouse CCI-ION pain model allows us to distinguish between an acute pain phase (3-5 days after injury) from a persistent pain phase driven by descending brain stem mechanisms (12-14 days after CCI-ION). CCI-ION induced significant decreases in mechanical pain thresholds in both the acute (72% decrease) and persistent (86% decrease) phases. To estimate chloride reversal potentials in laminae I/II neurons from trigeminal nucleus caudalis, we obtained whole cell recordings *in vitro*, using the gramicidin perforated patch clamp technique. We recorded from slices obtained from transgenic mice in which inhibitory, GABAergic neurons express GFP under the GAD65 promoter. We identified projection neurons by their content of fluorescent beads, retrogradely transported from the parabrachial nucleus. Chloride reversal potential decreased significantly during the acute phase (3 to 5 days), from -71±2mV in control animals to -65±4.7mV in CCI-ION animals. Projection neurons and unidentified neurons accounted for this change, whereas no significant changes occurred in GABAergic (GFP expressing) interneurons. In contrast, at 12 to 14 days after CCI-ION chloride reversal potential recovered to normal values (-70±2 mV in CCI-ION animals) in all types of neurons. Analysis of western blots of KCC2 revealed no statistically significant changes in protein expression at either 3 to 5 days or 12 to 14 days after CCI-ION, compared to control animals. These findings indicate that CCI-ION in mice results in transient and modest changes in chloride reversal potentials, and that these changes do not persist during the late pain phase. We suggest that in the mouse model of CCI-ION, changes in chloride reversal potential do not contribute to the central sensitization mechanisms leading to chronic pain.

Disclosures: A.M. Castro: None. **W. Guo:** None. **C. Raver:** None. **F. Wei:** None. **R. Dubner:** None. **A. Keller:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.09/U22

Topic: D.08. Pain

Support: NIH Grant DE024629

Title: Central role of calcitonin gene-related peptide in promoting peripheral sensitization of trigeminal nociceptive neurons

Authors: *L. CORNELISON, J. L. HAWKINS, C. HYDE, P. L. DURHAM;
Missouri State Univ., Springfield, MO

Abstract: Objective: To investigate the role of calcitonin gene-related peptide (CGRP) on the initiation and maintenance of a nocifensive withdrawal response to mechanical stimulation following activation of primary trigeminal sensory neurons. Background: Temporomandibular joint disorder (TMD) is characterized by peripheral and central sensitization of trigeminal nociceptive neurons. Although CGRP is implicated in the development of central sensitization by stimulating glial activation via its receptor, the mechanism by which CGRP promotes and maintains sensitization of trigeminal nociceptive neurons is not well understood. Methods: The direct role of CGRP was investigated in adult male Sprague Dawley rats following intrathecal injection of CGRP alone or co-injection with the CGRP receptor antagonist CGRP8-37. The effect of CGRP8-37 was evaluated in animals subjected to prolonged jaw opening to mimic pathological aspects of TMD. Nocifensive withdrawal response to mechanical stimulation of trigeminal nerves utilizing von Frey filaments was investigated daily up to 14 days post treatment. Cytokine levels were determined in upper spinal cord tissue using protein arrays. To provide evidence of bidirectional signaling in the trigeminal system, animals were co-injected intrathecally with CGRP and Fast Blue dye and fluorescent microscopy used to localize the dye in neuronal cell bodies within the trigeminal ganglion. Results: Intrathecal injection of CGRP and prolonged jaw opening increased nocifensive responses to mechanical stimulation up to 48 hours and up to 7 days, respectively, when compared to vehicle injection and naïve controls. Co-injection of the antagonist peptide CGRP8-37 with CGRP repressed CGRP's sensitizing effects on trigeminal nociceptive neurons. Similarly, injection with CGRP8-37 prior to prolonged jaw opening decreased the average number of nocifensive responses. CGRP and prolonged jaw opening promoted the expression of numerous cytokines in the spinal cord when compared to control levels. Staining for Fast Blue was observed in the cell bodies of trigeminal ganglion neurons following injection of the dye in the upper spinal cord. Conclusion: The ability of CGRP8-37 to repress nocifensive behavior supports the benefit of targeting CGRP or its receptor as a treatment strategy for diseases involving trigeminal activation. Our findings with respect to CGRP and Fast Blue support the notion of bidirectional signaling within the trigeminal system whereby changes within the spinal cord associated with central sensitization can facilitate a corresponding response within the trigeminal ganglion to promote peripheral sensitization.

Disclosures: L. Cornelison: None. **J.L. Hawkins:** None. **C. Hyde:** None. **P.L. Durham:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research

relationship even if those funds come to an institution.; DOD, Banyon Group, Allergan, IDF, GelStat.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.10/U23

Topic: D.08. Pain

Support: NIH EY021447

Title: Corneal reflexes in dry eye: evidence for altered amino acid transmission in trigeminal brainstem

Authors: K. SHIOZAKI[1], M. RAHMAN[2], K. OKAMOTO[2], R. THOMPSON[2], *D. A. BEREITER[2];
[1]Oral Anat., Tsurumi U Sch. of Dent. Med., Yokohama, Japan; [2]Diagnos. & Biol. Sci., U of Minnesota Sch. of Dent., Minneapolis, MN

Abstract: Corneal reflexes are evoked by ocular surface stimulation and serve to protect the eye and maintain the integrity of the tear film. The ocular surface is innervated by trigeminal sensory neurons that form the afferent limb of the corneal reflex and terminate in two spatially discrete regions of the trigeminal brainstem nuclear complex (TBNC), the subnucleus interpolaris/caudalis transition (Vi/Vc) and the caudalis/upper cervical cord junction (Vc/C1). Dry eye (DE) is a common ocular pain condition and is associated with increased spontaneous eye blinks and altered corneal reflexes. Although ocular surface input is integrated at the TBNC to mediate corneal reflexes, the effects of DE on brainstem mechanisms that underlie corneal reflexes are not well defined. The aim of this study was to determine if GABAergic and NMDA receptor mechanisms, acting at the Vi/Vc transition and Vc/C1 regions, affect corneal reflexes in a model for DE. Male rats were anesthetized (urethane) and corneal reflexes (orbicularis oculi activity, OOemg) were evoked by hypertonic saline (HS, 2.5M) 14d after exorbital gland removal, a model for tear-deficient DE. HS-evoked OOemg activity in DE rats was more than twice that of sham controls. Microinjection of the GABAaR agonist, muscimol (0.1-1mM, 0.3µl) into the Vi/Vc transition 10 min prior to HS caused a dose-related inhibition of OOemg and at a lower threshold dose in DE than sham rats, whereas injection into Vc/C1 inhibited OOemg similarly in both groups and at a higher dose. Microinjection of the NMDAr antagonist, AP5 (0.01-1mM, 0.3µl) into Vi/Vc also caused a dose-related inhibition of HS-evoked OOemg activity that was greater in DE than sham rats, whereas injection into Vc/C1 had a similar inhibitory effect in both groups. These results suggested that GABAergic and NMDA receptor mechanisms acting at the Vi/Vc transition and the Vc/C1 junction were necessary for corneal reflexes in sham rats. By contrast, GABAergic and NMDA contributions to corneal reflex

activity were enhanced at the Vi/Vc transition, but not at the Vc/C1 junction, in DE rats. These data support the hypothesis that altered amino acid transmission and synaptic plasticity of ocular neurons at the Vi/Vc transition contribute to abnormal corneal reflexes and sensations in DE.

Disclosures: K. Shiozaki: None. **M. Rahman:** None. **K. Okamoto:** None. **R. Thompson:** None. **D.A. Bereiter:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.11/U24

Topic: D.08. Pain

Support: CCOM Summer Fellowship (KAL)

MWU Intramurals (PFM)

Title: Innervation of the external nares: implications for initiation of the mammalian diving response

Authors: K. A. LAHRMAN, K. M. DINOVO, *P. F. MCCULLOCH;
Physiol., Midwestern Univ., Downers Grove, IL

Abstract: The purpose of this research was to determine the central projections of nerves innervating the external nares of rats that may be involved in initiating the autonomic cardiorespiratory reflex known as the diving response. The anterior ethmoidal nerve (AEN), a branch of the ophthalmic division of the trigeminal nerve that innervates the nose and external nares, is thought to provide the afferent signal that initiates the apnea, bradycardia, and selective increase in peripheral vascular resistance that occurs in response to underwater submersion. However recent research has indicated that rats retain the complete diving response even after bilateral AEN sectioning, suggesting that other nasal nerves can also provide the afferent signal to initiate this response. The transganglionic tracer Wheat Germ Agglutinin (WGA) was directly injected 5-10 mm into the left nasal passages of Sprague-Dawley rats (N=4). After immunohistological processing and fluorescent brainstem tissue visualization with a Nikon AR1 confocal microscope, central tracer terminations were found densely within the ventral tip of the left spinal trigeminal nucleus caudalis (medullary dorsal horn; MDH), especially along the transition with nucleus interpolaris; sparsely within the right MDH; bilaterally within the nucleus tractus solitarius (NTS); within the left dorsal trigeminal tract; and near to the left Botzinger complex. In other rats WGA was injected into the left nasal passages after the AENs had been sectioned bilaterally (N=4). Label in the ventral MDH was now less prominent and label near the Botzinger complex was absent, while label in the NTS was still present. WGA injected directly into the AEN (N=4) produced labeling within the ventral MDH, especially along the transition

with nucleus interpolaris, and near to the Botzinger complex, but no labeling within the NTS. WGA was also injected directly into the Infraorbital nerve (ION; N=7), a branch of the maxillary division of the trigeminal nerve that innervates the external nares and upper lip. Label after ION injection was found within the ventral MDH. Results indicate that the ION, in addition to the AEN, could possibly be an anatomical pathway that provides the afferent information necessary to initiate the mammalian diving response. Results also suggest that innervation of the nasal passages projecting to the NTS does not do so via the AEN or ION.

Disclosures: K.A. Lahrman: None. **K.M. DiNovo:** None. **P.F. McCulloch:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.12/U25

Topic: D.08. Pain

Support: Tsumura & Co

15K20377 Kakenhi

Title: Ginger-contained shogaol and gingerol inhibit oral ulcer-induced pain through sodium channel blockage

Authors: *S. HITOMI[1], K. ONO[1], K. YAMAGUCHI[1], K. TERAWAKI[2], Y. OMIYA[2], K. INENAGA[1];
[1]Kyushu Dent. Univ., Fukuoka, Japan; [2]Tsumura & Co, Ibaraki, Japan

Abstract: Recently, some clinical studies have demonstrated that the Japanese herbal medicine Hangeshashinto alleviates oral mucositis-induced pain in patients. Hangeshashinto contains seven herbal extracts, coptis rhizome, ginseng, glycyrrhiza, jujube, pinellia tuber, processed ginger, and scutellaria root. However, pharmacological mechanism underlying the analgesic effect of Hangeshashinto has not been investigated. Firstly, we selected 21 chemical ingredients in Hangeshashinto. In screening analysis systems (automated patch clamp recording and fluorescence imaging plate reader), the ingredients were evaluated in antagonistic and agonistic effects on nociception-related channels, a sodium channel Nav1.8 and transient receptor potential vanilloid 1 (TRPV1), respectively. Among the 21 ingredients, [6]-shogaol and [6]-gingerol showed strong antagonistic effects on Nav1.8, as the same as lidocaine. [6]-shogaol and [6]-gingerol also showed strong agonistic effects on TRPV1, as reported in previous studies. Therefore, we investigated analgesic effects of processed ginger and a mixture of [6]-shogaol and [6]-gingerol on pain-related behaviors in oral ulcer rat model treated by acetic acid. Processed ginger and the mixture of [6]-shogaol and [6]-gingerol did not change the mechanical pain hypersensitivity. However, by co-application with the other herbal extract the ginseng,

which abundantly contains saponins, these ingredients supressed the mechanical pain hypersensitivity. The ginseng extract accelerated cell membrane permeability of the fluorescence substance FluoroGold in the ulcer region where the epithelial barrier was destroyed. These results suggest that the oral ulcer-induced mechanical pain hypersensitivity is by antagonistic effect of [6]-shogaol and [6]-gingerol on sodium channels, via ulcer region with drug delivery acceleration due to other saponin-containing herbal extracts.

Disclosures: **S. Hitomi:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Tsumura & Co. **K. Ono:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Tsumura & Co. **K. Yamaguchi:** None. **K. Terawaki:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Kyushu Dental University. **Y. Omiya:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Kyushu Dental University. **K. Inenaga:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Tsumura & Co.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.13/U26

Topic: D.08. Pain

Title: Changes on the preference test for ethanol after mental nerve constriction in rats

Authors: *A. GARCIA URBINA[1], R. OCHOA MARTINEZ[2], R. MORALES DE LA LUZ[2], I. O. PEREZ-MARTINEZ[2];
[1]Univ. Nacional Autónoma De México, Tlalnepantla De Baz, Mexico; [2]FES Iztacala, Universidad Nacional Autónoma de México, Mexico

Abstract: Orofacial pain is a common and disabling problem. Despite current therapies, in the present research there is still a limited understanding of the nociceptive pathophysiology of pain. The aim of this research is the study of changes in the oral somatosensory perception because of trigeminal nerve injury. We assess the adaptive response to oral ethanol administration and the

modifications in this adaptation generated by the mental nerve constriction. We used two groups of male Wistar rats, they were water deprived for 23hours(h) previous each session. As baseline, the rats were given 3 days of water in their home cages every 23.5h for 15minutes(min), the volume consumption was registered. On days 4 to 8, the subjects were tested to response by the presentation of 8% ethanol solution for the group 1, and 20% ethanol solution for the group 2, for 15min. Then they received access to water for 15min. to ensure that all animals consumed their daily fluid requirement. To evaluate the subsequent motivation after the adaptation to ethanol consumption we use the two-bottle preference test (ethanol/water) before and after the trigeminal manipulation. Each rat was separated, and giving free access to the two bottles: water/ethanol (8%/20%, it depends on previous concentration used for each rat). The position of bottles was changed after 24h to prevent position preference. The avarage measure was obtained across 48h, and corrected for body weight of subjects. We found that the mental nerve transection produces changes in the perception of ethanol directly in the tongue. Differently to Augier et.al.(2014), in our project the ethanol 20% works like an aversive stimuli when it is presented at first (20% vs 8%, t test, p=0.04), however, the behavior is adapted regarding to persistent administration producing a gradually increasing of intake, we posit that the trigeminal system suffers molecular changes in free ends of the nerve, these changes could be the decreasing of the TRPV1 in the epithelial tissue of tongue. Furthermore, we found that the preference by ethanol decreases after mental nerve constriction in 20% (t test, p=0.001, water vs ethanol and t test, p=0.017, ethanol before surgery vs ethanol after surgery). The same by 8% of ethanol (t test, p=0.007, water vs ethanol and t test, p=0.19, ethanol before surgery vs ethanol after surgery). Comparisons between both water-intake before and after surgery not produces changes. We are showing the evidence that the injury in the mental nerve produces changes in the somatosensorial processing of ethanol during intake, these may be related to changes in the molecular characteristics of the trigeminal nerve ends.

Disclosures: A. Garcia urbina: None. **R. Ochoa Martinez:** None. **R. Morales de la Luz:** None. **I.O. Perez-Martinez:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.14/U27

Topic: D.08. Pain

Support: NIH Grant R01 ES017430

IU Collaborative Research Grant

Title: Sensitization of the trigeminovascular system following environmental irritant exposure in rodents: characterization of a novel model of chronic headache

Authors: P. E. KUNKLER[1], L. ZHANG[1], *G. S. OXFORD[2,1], J. H. HURLEY[1];
[1]Stark Neurosciences Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Indiana Univ. Sch. Med., Indianapolis, IN

Abstract: Headache is one of the most common neurological disorders, yet remains poorly understood and undertreated. Air pollution and environmental irritants have been associated with the initiation of headache however the neurobiological link between these chemical triggers and the induction of headache attacks remains unknown. Headache pain is thought to be caused by the release of inflammatory substances from trigeminal sensory nerve endings, resulting in neurogenic inflammation and vasodilation in the dura. Supporting this notion, we previously reported that acute nasal administration of environmental irritants increases meningeal blood flow via a TRPA1-dependent mechanism involving the trigeminovascular system. In addition, repeated inhalation exposure of subacute doses of acrolein potentiated blood flow responses to both TRPA1 and TRPV1 agonists suggesting trigeminovascular sensitization as the mechanism for enhanced headache susceptibility. We hypothesize that the nasal-to-meningeal neuronal pathway activating the trigeminovascular response involves trigeminal intraganglionic signaling, as we observed that nasal and meningeal nerve terminals arise from adjacent but distinct soma in the trigeminal ganglion. Also, inhibition of NMDA and/or CGRP receptors only in the trigeminal ganglion compromised the nasal-to-meningeal responses, implicating intraganglionic transmission. Here we investigated behavioral, anatomical and trigeminovascular responses induced by repeated acrolein inhalation exposure to further characterize the effect of environmental irritants on the trigeminovascular system. Laser Doppler flowmetry confirmed that daily repeated acrolein exposure significantly ($p < 0.05$) potentiated meningeal blood flow responses to acute nasal administration of mustard oil compared to room air controls ($n = 8$ per group). Periorbital allodynia tests show that repeated acrolein exposure significantly enhanced cutaneous mechanical sensitivity ($p < 0.05$) both immediately following the last inhalation exposure and 24 hrs later. Enhanced cutaneous allodynia was further confirmed with touch-induced c-Fos expression. c-Fos staining in the spinal trigeminal nucleus of rats repeatedly exposed to acrolein is significantly increased compared to room air controls. Taken together these results demonstrate trigeminovascular sensitization following environmental irritant exposure. This novel model may allow us to gain a better understanding of migraine triggers and perhaps the progression to chronic migraine.

Disclosures: P.E. Kunkler: None. **L. Zhang:** None. **G.S. Oxford:** None. **J.H. Hurley:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.15/U28

Topic: D.08. Pain

Support: International Dehydrated Foods

Title: Chicken broth aac1 as a dietary supplement represses nocifensive behaviors and pka expression caused by prolonged jaw opening

Authors: *J. L. HAWKINS, P. L. DURHAM;
JVIC/CBLS, Missouri State Univ., Springfield, MO

Abstract: Objective: The goal of this study was to determine if the dietary inclusion of the commercially available chicken broth AAC1 could repress nocifensive behaviors and PKA expression associated with a clinically relevant model of temporomandibular joint disorder (TMD) caused by prolonged jaw opening. Background: Routine dental visits for molar extractions or root canals, or to the orthodontist can result in an injury to the TMJ or muscles of mastication if the jaw is held open for long periods of time or is opened to near maximal even for a short period of time. However, the pathophysiological and cellular mediators that underlie the development of chronic orofacial pain associated with TMD are not well understood. Chicken broth has been often thought of as having beneficial immune effects and may represent an innovative and safe alternative treatment for chronic inflammatory diseases. Methods: Male Sprague-Dawley rats were used to investigate the effects AAC1 on behavioral and cellular changes associated with prolonged jaw opening. A surgical retractor was placed around the bottom and top incisors and the jaw held at near maximal opening for 20 minutes. Withdrawal responses to mechanical stimuli were determined following jaw opening for up to 7 days by applying calibrated von Frey filaments to the cutaneous area over the masseter. Some animals received 0.5% solid (w/v) AAC1 dissolved in their drinking water for two weeks prior to jaw opening. After 7 days, animals were euthanized and the upper spinal cord and trigeminal ganglia were removed to study changes in the expression of PKA utilizing immunohistochemistry. Results: Near maximal jaw opening was sufficient to induce sustained increased nocifensive responses to mechanical stimuli over the masseter area for 7 days. This increased sensitivity correlated with increased levels of PKA expression in both the upper spinal cord and the trigeminal ganglia. Interestingly, dietary inclusion of AAC1 was able to suppress nocifensive behaviors and PKA expression associated with prolonged jaw opening. Conclusion: Our findings provide evidence that dietary inclusion of chicken broth AAC1 suppresses mechanical hyperalgesia and increased PKA expression associated with peripheral and central sensitization of the trigeminal system and thus, may by a potential nutraceutical supplement that may help manage inflammatory pain associated with TMD.

Disclosures: J.L. Hawkins: None. **P.L. Durham:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; DOD, Banyon Group, Allergan, IDF, GelStat.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.16/U29

Topic: D.08. Pain

Support: NIH Grant P41 EB015894

NIH Grant P30 NS076408

Title: A three dimensional assessment of 7T tractography of the trigeminal nerve through anatomical dissection

Authors: *A. M. EL-NASHAR[1,2], J. SEIN[5], J. TOKAREV[3], B. JAGADEESAN[4], P.-F. VAN DE MOORTELE[3], A. W. GRANDE[2], C. LENGLET[3];
[2]Dept. of Neurosurg., [3]Ctr. for Magnetic Resonance Res., [4]Dept. of Radiology, [1]Univ. of Minnesota, Minneapolis, MN; [5]Univ. of Aix-Marseille, Marseille Area, France

Abstract: The anatomy of the trigeminal nerve fiber tracts is complex. In the brain stem, tracts arise from four separate nuclei located between the mesencephalon superiorly, and the cervical cord inferiorly. At mid-pons level, emerging fibers twist in the prepontine cistern, enter Meckel's cave, form the Gasserian ganglion and finally divide into three main divisions [1]. Ultra high field MRI techniques (using systems 7 Tesla and above) enable accurate in-vivo examination of the fiber tracts [2] including both the motor fibers and the sensory fibers within the three main divisions. In this study we validate the data obtained using these techniques by comparing them with the data from human cadaver fiber tract dissection. Healthy volunteers were scanned using a 7T MRI system (Siemens, Erlangen, Germany) equipped with a volume transmit, and a 24-channel receive head coil (Nova Medical, Inc. Wilmington, MA, USA). Diffusion MRI was acquired with voxel size=1.2x1.2x1.2 mm3 (reconstructed at 0.6x0.6x1.2 mm3 with zero filling), 18 slices, TR/TE=5000/64 ms, 100 gradient directions, b-value=1000 s/mm2, 11 b=0 volumes. Following correction for distortions and head motion, and fiber orientation mapping [3], we placed seeding masks in the floor of the fourth ventricle and the cisternal segment of the trigeminal nerve. We employed probabilistic tractography using FMRIB Software Library to identify fibers within the trigeminal nerve pathway. Separately, white matter fiber dissection technique was used to study the microsurgical anatomy of formalin-fixed brainstems from human cadavers. The generated fiber tracts, using diffusion MRI and tractography, were visualized in three dimensional space using Amira, and compared to the tracts obtained from fiber dissection. Trigeminal nerve sensory and motor pathways from the brain stem out to the trigeminal divisions were visualized as reproducibly distinct structures from one another in the 7T data and highly correlated to anatomic fiber dissection. However, limitations with the 7T data were seen when assessing the topographic representation within the brain stem, where there was a limited correlation with the fibers obtained from dissection. 7T diffusion MRI produces reliable imaging of the trigeminal fiber tracts when compared to anatomic dissection. Clinically, this enables us to study the pathophysiology of trigeminal neuralgia from neurovascular conflict as it may relate to the observed frequency of involvement of the individual divisions in patients with

trigeminal neuralgia. References: [1] Joo et al., Clinical Anat., 2014. [2] Lenglet et al., ISMRM, 2014. [3]Behrens et al, Neuroimage, 2007.

Disclosures: **A.M. El-Nashar:** None. **J. Sein:** None. **J. Tokarev:** None. **B. Jagadeesan:** None. **P. Van de Moortele:** None. **A.W. Grande:** None. **C. Lenglet:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Partly supported by NIH grants P41 EB015894 & P30 NS076408..

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.17/U30

Topic: D.08. Pain

Support: JPSP KAKENHI 25462908

Title: Local KOR agonist reduces TMJ-evoked activity of trigeminal subnucleus caudalis neurons in an estrogen-dependent manner

Authors: *A. TASHIRO, Y. NISHIDA;
Natl. Def. Med. Col., Tokorozawa, Saitama, Japan

Abstract: Painful temporomandibular joint disorders (TMD) occur more often in women than men and are difficult to treat. Sex difference in kappa opioid analgesia have been reported under a variety of test conditions in animal and human studies; however, the influence of estrogen (E2) on kappa opioid receptor (KOR) of nociceptive processing related to craniofacial pain is not well defined. To address this issue, single temporomandibular joint -responsive neurons (TMJ neuron) were recorded in laminae I-II at the spinomedullary (Vc/C1-2) junction from ovariectomized female rats (OvX) treated for 2 days with high E2 (20μg/day; HE2) or low E2 (2μg/day; LE2) under isoflurane anesthesia. TMJ neurons in Vc/C1-2 region were activated by test injections of ATP (1mM, 20μl) through a cannula placed in the TMJ joint space. The KOR agonist, U50488 (30-300μM) was applied topically to Vc/C1-2 surface 10 min before test injections of ATP. Topical U50488 caused a doses related inhibition of ATP-evoked unit activity in HE2 rats ($P < 0.01$), while units in LE2 rats displayed inconsistent effects. BNI (selective KOR antagonist) caused at least partial reversal of kappa opioid inhibition. U50488 also reduced significantly ATP-evoked response duration of units from HE2 ($P<0.01$), but not LE2. KOR activation had only minor effects on spontaneous unit activity of TMJ units in OvX female rats (HE2 and LE2). High dose U50488 caused small reduction in size of convergent cutaneous receptive field in HE2 rats, but not LE2 rats. These results indicated that estrogen status

differentially affected KOR agonist-modulation of TMJ unit activity in superficial laminae at Vc/C1-2 junction in female rats. The site for estrogen influence on KOR agonist-induced modulation of TMJ unit activity was the medullary dorsal horn. It is concluded that selective activation of KOR in medullary dorsal horn produce estrogen dependent attenuation of TMJ pain.

Disclosures: A. Tashiro: None. **Y. Nishida:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.18/U31

Topic: D.08. Pain

Support: NIH DE017805

Title: Persistent sensitization of trigeminal neurons promotes gut dysbiosis

Authors: *P. L. DURHAM, J. L. HAWKINS, R. NORTON;
JVIC-CBLS, Missouri State Univ., Springfield, MO

Abstract: Objective: To investigate changes in the gut microbiota that promotes an imbalance or dysbiosis in response to prolonged sensitization of primary trigeminal sensory neurons. Background: Diseases involving the head and face represent some on the most common pain sites in the body. Neck muscle pathology and sleep deprivation are reported as risk factors by orofacial pain patients. Another pathological condition often associated with orofacial pain is irritable bowel syndrome (IBS), which is characterized by dysbiosis. Understanding the relationship between increased nociceptive signaling in the trigeminal system and the gut microbiota is essential to fully understand the gut-brain axis and subsequent negative health outcomes associated with gut dysbiosis. Materials: Sprague-Dawley rats were injected with complete Freund's adjuvant (CFA) in the trapezius to cause prolonged inflammation and REM sleep deprived for 24 hours. Nocifensive behavioral changes were monitored for up to 14 days post CFA injection using von Frey filaments. Fecal samples were collected from each animal at day 0 and day 14. Total DNA was extracted and amplified from the V3-V4 region of the 16S rRNA gene with sample-specific barcode sequences prior to 454-pyrosequencing. Returned sequences were assigned taxonomic classification using the SILVA rRNA database. Microbial community analysis was performed using phylogenetic packages in R. Results: Animals with neck muscle inflammation and also REM sleep deprived exhibited a sustained sensitivity to mechanical stimuli (nociception) that temporally correlated with significant changes in the composition of the gut microbiota. Sequence analysis revealed large-scale shifts in the phyla Bacteroidetes and Proteobacteria, including Bacteroides species that have been implicated in

intestinal and organismal health. Prolonged sensitization resulted in a loss of commensal bacteria and the emergence of bacteria not abundantly seen in healthy animals. Conclusion: Our results provide evidence that inflammation in the trapezius followed by one night of REM sleep deprivation is sufficient to induce sustained sensitization of trigeminal neurons and dysbiosis along the gut-brain axis. Based on our findings, we propose that sensitization of the neurons associated with orofacial pain changes the composition of the gut microbiota, and thus our results may help to explain the reported high co-morbidity of chronic orofacial pain and IBS. Study Supported by: NIH DE017805.

Disclosures: **P.L. Durham:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GelStat; IDF;Allergan; DOD, Banyon Group. **J.L. Hawkins:** None. **R. Norton:** None.

Poster

336. Trigeminal Processing

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 336.19/U32

Topic: D.08. Pain

Support: Grant-in-Aid for Scientific Research（KAKENHI) 26463181

Title: The dry eye sensitizes cold cell sensitivity to capsaicin mediated by TRPV1

Authors: ***M. KUROSE**[1], A. HATTA[1], K. YAMAMURA[1], I. D. MENG[2];
[1]Niigata Univ. Grad. Sch. of Dent., Niigata, Japan; [2]Dept. of Biomed. Sci., Col. of Osteo. Medicine, Univ. of New England, Biddeford, ME

Abstract: Previous studies have found that cold cells innervating the cornea are sensitive to the ocular fluid status of the corneal surface and may be responsible for the regulation of basal (ongoing) tear production. We had focused on the encoding property of cold cells, and shown the facilitation of cold-evoked activity after application of TRPM8 agonist menthol and suppression of cold-evoked activity after application of TRPV1 agonist capsaicin. In addition, we have shown that an experimental dry eye condition produced by the lacrimal gland excision modifies the thermal and menthol responses in these neurons. In the present study, we examined the effect of dry eye on the sensitivity of cold cells to the capsaicin. Forty-five male SD rats weighing 200-225 g at the time of surgery were used. Under isoflurane anesthesia, unilateral dry eye was created by excision of the left exorbital and infraorbital lacrimal glands. Extracellular, single-unit recordings were performed in urethane-chloralose anesthetized animals 1 week after lacrimal gland excision and in age matched controls. Electrodes positioned in the trigeminal ganglion were used to isolate and characterize cold-sensitive corneal neurons. Responses to thermal

stimulation were examined 5 min after the application of capsaicin (3 nM- 3 μM) and its vehicle. At low concentrations (<300 nM), capsaicin did not affect cold cell activity in control animals; in contrast, ongoing activity was facilitated and cold-evoked activity was significantly suppressed in dry eye animals. High concentrations of capsaicin (≥300 nM) suppressed the ongoing and cold-evoked activity in both groups of animals, with an overall greater suppression in dry eye animals. We applied the TRPV1 antagonist, capsazepine (10 μM) 30 min before capsaicin application in dry eye animals. Capsazepine blocked the capsaicin-induced modulation of cold cell activity. These results indicate that dry eye sensitizes cold cells to capsaicin-mediated inhibition via TRPV1.

Disclosures: **M. Kurose:** None. **A. Hatta:** None. **K. Yamamura:** None. **I.D. Meng:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.01/U33

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant NS048425

Title: Somatosensory and motor corticospinal projections respond differently to peripheral and central spinal injuries in monkeys

Authors: ***C. J. DARIAN-SMITH**[1], K. M. FISHER[1], A. L. LILAK[1], K.-A. IRVINE[2], J. GARNER[1];
[1]Comparative Med., Stanford Univ. Sch. of Med., Stanford, CA; [2]Dept. of Neurol., UCSF and SFVAMC, San Francisco, CA

Abstract: The primate corticospinal tract (CST) originates from >9 functional regions of the neocortex, and forms the major descending pathway involved in voluntary hand movements. We have previously shown that these discrete CST subcomponents respond quite differently to different spinal injuries affecting sensory pathways. Thus, following a cervical dorsal rhizotomy (DRL), terminal projections from the primary somatosensory cortex (S1) shrink to 60% of normal, while the motor CST projection territory remains robust and even expands (Darian-Smith et al., 2013, JCN 521:2359-72). The motor CST is therefore dominant in circuitry remodeling following a DRL alone. In contrast, when a DRL is combined with a cervical DCL (dorsal cuneate fasciculus at C5), both the S1 and motor CSTs respond dramatically (Darian-Smith et al., 2014 JNeurosci 34:12267-12279), sprouting bilaterally and caudally (>2cm) well beyond their normal terminal distribution territories. While these data indicate that the central injury (the DCL) and associated immune response are critical to the dramatic S1 and motor CST outgrowth, it was not clear if the DCL alone was responsible, or if there was some combinatorial

effect; we addressed this issue directly in the present study. Monkeys from our previous investigations (above), that had received either a DRL (involving C5-C8 rootlets innervating just the thumb (D1), index (D2) and middle fingers (D3)), or a DRL/DCL, were compared with two animals receiving a DCL alone (cuneate fasciculus at C5 or C6). Three months following the DCL lesion, electrophysiological recordings were made within the somatosensory cortex to identify the reorganized region of D1-D3 representation. Anterograde tracers (LYD, BDA) were then injected bilaterally into the reorganized cortex (S1 and primary motor), to assess axon terminal distribution patterns in the cervical and thoracic cord. Preliminary data have been analyzed from one of two monkeys. In this animal, terminal projections from S1 were found to extend well beyond their normal range, occupying the intermediate zone bilaterally from C1-rostral T1, in addition to the dorsal horn (C5-T1). Sparse projections were also observed within the ventral horn in C5-C8. Motor CST terminations were also robust, and more extensive than in normal animals. In contrast to animals with a combined DRL/DCL, neither the motor nor the S1 CSTs sprouted caudally beyond their normal range. Our preliminary findings suggest a combinatorial effect of the two lesions on CST outgrowth. We will present our final analysis, including a multifactorial statistical comparison of the changes following the three different lesions (a DRL, DRL/DCL, and a DCL).

Disclosures: C.J. Darian-Smith: None. **K.M. Fisher:** None. **A.L. Lilak:** None. **K. Irvine:** None. **J. Garner:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.02/U34

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Effect of trasplant of pedegenerated peripheral nerve and bone marrow stromal cells over axonal regeneration and remyelinitation in a rat model of transection spinal cord injury

Authors: *V. BUZOIANU ANGUIANO[1], S. OROZCO-SUAREZ[2], S. CABALLERO-CHACÓN[3], I. GRIJALVA[2];
[1]Home, Mexico City, Mexico; [2]Unidad De Investigation Medica En Enfermedades Neurologicas, Insitituto Mexicano Del Seguro Social, Mexico City, Mexico; [3]Department De Farmacologia De Y Fiologia, Facultad De Medicina Veterinaria Y Zootecnia, Universidad Nacional Autonoma De Mexico, Mexico City, Mexico

Abstract: Recovery of the spinal cord (SC), is limited because of the low cell regeneration and axonal growth, also due to the failure of replacement of damaged myelin, which prevents functional recovery of neuronal connections. Some treatments such as predegenerated peripheral nerve transplantation (PPN) and bone marrow stromal cells (BMSc) have shown to promote

axonal regrowth as well as remyelinitation of central and collateral SC axons in complete section model. It has shown that there is the presence of proteins such as GAP-43, Neuritin and myelin basic protein PBM which favor a permeable micro-environment for axonal regrowth and remilinizacion after a TCSI. The aim of this study was to determine the expression of these proteins in animals with transplants of PPN and PPN+BMSc after a chronic transection of the spinal cord. Thirty eigth female Fisher 344 rats were subjected to a whole spinal cord transection at thoracic 9 (T9); after four weeks of the evolution, they were ramdomly distributed in 4 groups, group 1 (TSCI without treatment); group 2 (TSCI+Fibrin Glue); group 3 (TSCI+ Fibrin Glue+ PPN transplantation) and group 4 (TSCI+ Fibrin Glue+ PPN transplantation + BMSc transplantation). After eight weeks post-trasplant, the animals were sacrificed and spinal cord was obtained by histological study; areas proximal and distal to the site of the transplant were prepared to determine the expression of GAP-43, Neuritin and PBM proteins by fluorescence density. Results: GAP-43 and Neuritin expression both proximal and distal stumps of groups 3 and 4 were higher than group 1 and 2 ($P< 0.05$). PBM expression of proximal stump was also higher in groups 3 and 4 than groups 1 and 2 ($P < 0.05$); moreover, it was seen higher expression in distal zone of 4 than group 3 ($P< 0.001$). In conclusión: PPN and BMSc transplantation promote axonal regrowth and remyelination in a rat model of chronic SCI transection.

Disclosures: V. Buzoianu anguiano: None. **S. Orozco-suarez:** None. **S. Caballero-Chacón:** None. **I. Grijalva:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.03/U35

Topic: D.10. Spinal Cord Injury and Plasticity

Support: The WalkAbout Foundation

Christopher & Dana Reeve Foundation

Title: Use of peripheral nerve stimulation to locate interneurons associated with group i and group ii afferents in intact mouse spinal cords

Authors: B. N. PHAM, Jr.[1], H. ZHONG[2], R. ROY[2,3], *N. J. TILLAKARATNE[2,3], V. EDGERTON[2,5,4,3];
[1]Bioengineering, [2]Dept Integrative Biol. and Physiol., [3]Brain Res. Inst., [4]Neurosurg., UCLA, Los Angeles, CA; [5]Ucla, Neurobio., Los Angeles, CA

Abstract: The role of proprioceptive input on locomotor recovery after a spinal cord injury (SCI) has been shown behaviorally and electrophysiolgically, but more work needs to be done at the cellular and network levels. Group I and Group II afferents can be recruited selectively by

stimulating peripheral nerves at varying intensities above motor threshold. In a previous study, Group I afferents were recruited when different muscle nerves were stimulated at 1.5-1.8X motor threshold whereas Group II afferents were recruited at 2.5X motor threshold. To identify neurons associated with Group I or Group II afferents, we stimulated the isolated soleus nerve of intact C57Bl6 adult mice at either 1.5 or 3.5X motor threshold, respectively. In addition, the isolated soleus nerve also was stimulated at 3.5x motor threshold in a group of mice whose spinal cords were completely transected at a mid-thoracic level. All muscles of the upper and lower leg were denervated to isolate the soleus nerve: the stimulating electrode was placed on the distal portion of the soleus nerve. After 20 min of stimulation, the mice were maintained in a deep anesthetic state for 60 min (to maximize c-fos expression) and then perfused with paraformaldehyde (intracardially). The lumbar spinal cords were removed and cryosectioned (30-µm thick) and immunostained for c-fos (an activity marker). Intact mice stimulated at 3.5X motor threshold showed activated neurons throughout laminae I-VIII and X, with the majority localized in the ipsilateral dorsal horn. Intact mice stimulated at 1.5X motor threshold had less neuronal activation than those stimulated at 3.5X, but showed a similar laminar distribution. Mice stimulated at 3.5X threshold also showed a higher percentage of Fos^{+} cells in ipsilateral laminae IV-VIII and in the lateral funiculus. Spinal mice stimulated at 3.5X motor threshold showed less activation of neurons compared to intact mice stimulated at 3.5X motor threshold, but similar to that observed in intact mice stimulated at 1.5X motor threshold. These results show when the soleus nerve is stimulated the spinal neurons that process different types of afferent input have unique locations within the spinal grey matter and that a SCI decreases the excitability of these spinal interneurons.

Disclosures: B.N. Pham: None. **H. Zhong:** None. **R. Roy:** None. **N.J. Tillakaratne:** None. **V. Edgerton:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.04/U36

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant R01EB007615

NIH Grant 8P30GM103507

Christopher and Dana Reeve Foundation

Kessler Foundation

Leona M. and Harry B. Helmsley Charitable Trust

Kentucky Spinal Cord Injury Research Center

University of Louisville Foundation

Title: Activity-dependent improvement of full weight-bearing standing with epidural stimulation in chronic complete paraplegics

Authors: *E. REJC, C. ANGELI, S. HARKEMA;
Kentucky Spinal Cord Injury Res. Ctr., Univ. of Louisville, Louisville, KY

Abstract: Clinically complete spinal cord injury (SCI) is associated with the inability to stand or walk, and a drastic decrease in quality of life for affected individuals. The mammalian lumbosacral spinal cord can regain the ability to generate some level of weight-bearing standing after a complete SCI. Activity-dependent rehabilitation alone allowed the recovery of weight-bearing standing and stepping in complete spinal cats, while its interaction with lumbosacral spinal cord epidural stimulation was needed to achieve similar results in complete rats. The aim of this study was to investigate the effects of stand training with epidural stimulation in four clinically complete SCI individuals, who were implanted with an epidural electrode array over the segments L1-S1 of the spinal cord. EMG, kinematics and ground reaction forces were recorded during standing experimental sessions performed before and after 80 stand training sessions (1 hour of standing per session, 5 days/week). Before training, all four participants needed external assistance at hips and knees to maintain upright posture, even in the presence of epidural stimulation. EMG pattern of several lower limb muscles often consisted in the alternation between EMG bursts and periods of little activity, resulting in overall unstable assisted standing. Throughout the training, epidural stimulation parameters were optimized and all participants achieved full weight-bearing standing without external assistance for knee extension. The longest knee-independent standing bout achieved by each participant was substantially different, ranging from 4.3 (participant B07) to 63.0 minutes (participant A45). After training, EMG patterns were overall more continuous, and lower levels of external assistance were needed to stand with the same stimulation parameters tested before training. However, stable standing with the least amount of assistance was achieved with individual-specific stimulation parameters optimized during training. On the other hand, without stimulation, little or no EMG activity was recorded from the analyzed muscles of all research participants, who maintained upright posture because of the trainers' assistance at the knees and hips, and because of the weight-bearing action performed by their upper limbs. Part of the standing ability improvement after training can be explained by the enhanced motor output promoted by the reinforcement of the neural pathways directly and repetitively involved during stand training. However, the appropriate selection of stimulation parameters is critical to further improve standing after chronic complete paralysis in humans.

Disclosures: E. Rejc: None. **C. Angeli:** None. **S. Harkema:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.05/U37

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NJCSCR14

Title: Multi muscle neuromuscular electrical simulation of the lower limbs: Effect on intra and inter limb motor pools

Authors: *G. F. FORREST[1], M. BUGRAHAN BAYRAM[2], R. PILKAR[2], A. RAMANUJAM[2], M. MITCHELL[2], E. GARBARINI[2];
[1]Kessler Fndn. Res. Ctr., West Orange, NJ; [2]Human Performance and Engin. Lab., Kessler Fndn., West Orange, NJ

Abstract: Acute spinal cord injury often leads to rapid muscle atrophy in the paralyzed limbs. Recently, we have shown that an intense novel form of standardized multi-muscle neuromuscular electrical stimulation (NMES) combined with dynamic standing retraining tasks may potentially restore muscle structure and function after sub acute to chronic, motor-complete SCI. Specifically, we have presented data for a large number of standardized repetitive task specific training sessions of multi-muscle NMES of the lower limbs combined with mechanical loading to demonstrate an increase in bilateral muscle volume in conjunction with a significant increase in flexor and extensor muscle activation amplitude during continuous stepping. Albeit the phasic coordination of the flexor and extensor muscle activation pools were inappropriate for stepping. However, very little is known about the effect of multi-muscle NMES during mechanical loading on the motor pools of the ipsilateral and contralateral flexors or extensors during stimulation. We will present data on a series of experiments to illustrate intra and inter limb neuromuscular response for several individuals who have cervical motor complete SCI as well as several able bodied controls undergoing a standardized NMES and mechanical loading testing protocol. These data show that during repeated bouts of a ramping stimulation protocol to the lower limb extensor muscles while standing there was concomitant recorded stimulation to multiple muscle sites of contralateral limb. For both motor complete SCI and able bodied controls, there were significant increases in muscle activation amplitudes for both ipsilateral and contralateral extensors during NMES ramping protocol. Moreover these data reflected changes in spatial and temporal characteristics of the standardized repeated bouts of NMES train as well as the changes in the ipsilateral and contralateral loading forces and net center of pressure. We propose that our experiments potentially demonstrate that for the intact human spinal cord and for the motor complete individual, single muscle stimulation and multi-muscle stimulation may play a significant role in intra/inter limb neural circuitry changes.

Disclosures: G.F. Forrest: None. **M. Bugrahan Bayram:** None. **R. Pilkar:** None. **A. Ramanujam:** None. **M. Mitchell:** None. **E. Garbarini:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.06/U38

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant R01 HL96750

Title: Impact of BDNF/TrkB signaling on functional recovery across motor behaviors after cervical spinal cord injury

Authors: *H. M. GRANSEE[1], C. B. MANTILLA[1,2], Y. WANG[1], V. HERNANDEZ-TORRES[1], W.-Z. ZHAN[1], G. C. SIECK[1,2];
[1]Physiol. & Biomed. Engin., [2]Anesthesiol., Mayo Clin., Rochester, MN

Abstract: A C_2 cervical spinal cord hemisection (SH) disrupts descending inspiratory-related drive to phrenic motoneurons, paralyzing the ipsilateral diaphragm muscle. There is gradual recovery of rhythmic diaphragm muscle activity ipsilateral to injury over time, consistent with neuroplasticity and strengthening of spared synaptic inputs to phrenic motoneurons. Phrenic motoneurons are recruited across a range of motor behaviors to generate varying levels of diaphragm muscle force. Intrathecal delivery of brain-derived neurotrophic factor (BDNF) near the level of phrenic motoneurons enhances recovery of ipsilateral diaphragm activity after SH during eupnea. We hypothesized that intrathecal BDNF enhances diaphragm activity across both ventilatory and non-ventilatory motor behaviors after SH. An intrathecal catheter was placed in Sprague-Dawley rats at C_4 to chronically infuse artificial CSF (aCSF) or BDNF (180 ng/day) using an osmotic pump. Diaphragm EMG electrodes were implanted bilaterally to record activity across motor behaviors, i.e., eupnea, hypoxia-hypercapnia (10% O_2 and 5% CO_2), deep breaths, sustained airway occlusion, and sneezing. Functional recovery during eupnea at 14D was evident in 100% of BDNF treated rats and 38% of aCSF treated rats. During eupnea and hypoxia-hypercapnia, root mean square (RMS) EMG amplitude at 14D after SH was significantly greater after BDNF treatment compared to aCSF treatment. Diaphragm RMS EMG amplitude during sneezing was also greater after BDNF treatment compared to aCSF treatment; however, BDNF had no effect during deep breaths or sustained airway occlusion. Enhancing BDNF/TrkB signaling at the level of the phrenic motoneuron pool via intrathecal infusion is sufficient to promote recovery of diaphragm activity during ventilatory behaviors but not during all non-ventilatory behaviors.

Disclosures: H.M. Gransee: None. **C.B. Mantilla:** None. **Y. Wang:** None. **V. Hernandez-Torres:** None. **W. Zhan:** None. **G.C. Sieck:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.07/U39

Topic: D.10. Spinal Cord Injury and Plasticity

Support: DOD (SC110169)

GM103507

Kentucky Spinal Cord and Head Injury Research Trust (DSKM)

Title: Electromyographic patterns in the contralateral limb in response to muscle stretch in rats with moderate spinal cord injuries

Authors: *A. KELLER[1], K. NORD[2], A. WADE[2], A. SHUM-SIU[3], D. S. K. MAGNUSON[3]; [2]Bioengineering, [3]Neurolog. Surgery, [1]Univ. of Louisville, Louisville, KY

Abstract: Stretching remains a leading technique utilized by physical therapists to manage spasticity and contractures in patients with spinal cord injuries (SCI). Previously, we found that stretching negatively impacts locomotor recovery in rats with mild acute thoracic SCIs as well as in animals with moderately-severe acute and chronic SCIs. Clinical relevance of the stretching phenomenon is currently not known. Using electromyography (EMG), the goal of the present study was to characterize patterns of muscle responses to stretching that could provide a potential translational cue. Eight female Sprague-Dawley rats were used for this study (stretch EMG group n=4, non-stretch non-EMG controls, n=4). Stretch EMG group animals were instrumented with implantable telemetry-based transmitters connected to EMG electrodes (DSI). The body of the transmitter was placed subcutaneously on the back between the shoulder blades while EMG electrodes were inserted into the belly of the right knee flexors (Biceps Femoris) and extensors (Rectus Femoris). Twenty days later animals received moderate contusion (12.5 g/cm, NYU) SCIs at T10. Starting two weeks post-injury the left limb was taken through a 12 minute stretching protocol twice a week while EMG responses of the contralateral limb were recorded. In addition, we measured the forces that the rat "physical therapists" applied during stretching using a custom designed "force glove" and LabView software. We collected kinematics of the limb position in order to calculate the torques applied during stretching. Recurring EMG patterns in the right limb in response to stretching include spasms characterized by low to medium amplitude co-activation of flexors and extensors and clonus-like activity characterized by medium amplitude bursts at 4-6Hz. These responses occurred in the contralateral limb that was not being manipulated or touched in any way. Conclusions: We observed at least two responses, clonus and spasms, in our animals in response to muscle stretch that commonly occur in chronic incomplete SCI patients. In order to draw further implications about possible negative effects of stretching in human subjects, however, stretching studies in patients need to be conducted using similar protocols and outcome measures.

Disclosures: **A. Keller:** None. **K. Nord:** None. **A. Wade:** None. **A. Shum-Siu:** None. **D.S.K. Magnuson:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.08/U40

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH R01- NS079751

Title: Sympathetic-somatomotor coupling is disrupted in spinal cord injury

Authors: ***T. ONUSHKO**[1], T. G. HORNBY[2], B. D. SCHMIT[1];
[1]Biomed. Engin., Marquette Univ., Milwaukee, WI; [2]Rehabil. Inst. of Chicago, Chicago, IL

Abstract: The regulation of the cardiovascular system during muscle activity is poorly understood in people with incomplete spinal cord injury (SCI). While previous research has looked at sympathetic and somatomotor systems separately, changes in the interactions between these systems may affect motor output. Thus, the purpose of this study was to understand how the sympathetic system interacts with the somatomotor systems below the level of injury in people with incomplete SCI. We hypothesized the interaction between sympathetic and somatomotor systems is disrupted in people with incomplete SCI. We recruited 4 motor-incomplete SCI subjects (ASIA classification D; injury level above T1) and 9 healthy age-matched adults. Subjects were seated upright and their right leg strapped into an instrumented chair. The right knee was flexed 90° and aligned with a 6 degree of freedom load cell. We tested sympathetic function using 3 different stressors: 1. cognitive stressor (counting backwards by 13,), 2. cold pressor test (CPT; foot immersed in ~3° C ice water), and 3. pain (100 Hz electrical stimulation applied near the naval), while simultaneously monitoring changes in patellar tendon tap reflexes, maximal voluntary knee extension, and cardiovascular parameters. The sympathetic stressors were applied during the first 3 minutes of a 6-minute trial, and we continued to monitor changes for 3 additional minutes after the stressor was removed. Somatomotor measurements include electromyograms (EMG) and knee torque. Measurements of sympathetic function include heart rate, blood pressure and femoral artery blood flow using Doppler ultrasound. Measurements were made before the stressor was applied (baseline) and then every 1 minute during the 6-minute trial. Preliminary results show that the reflex responses to the sympathetic stressors differed between incomplete SCI and healthy adults. During the CPT, the reflex EMG responses increased in SCI subjects and remained elevated compared with healthy adults. We observed no trends in maximal knee extension torque during any of the conditions. We also observed differences in cardiovascular responses between SCI and healthy controls. The heart rate in SCI subjects decreased in response to the sympathetic stressors, while blood flow of the

femoral artery increased in response to pain and cold, which was the opposite compared with the healthy adults responses. Additionally, we observed a dulled response in mean arterial pressure in SCI subjects compared with the healthy adults. These data demonstrate that motor responses to sympathetic stressors is altered in people with incomplete SCI. Supported by NIH R01-NS079751

Disclosures: **T. Onushko:** None. **T.G. Hornby:** None. **B.D. Schmit:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.09/U41

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH grant R01 HL96750

Title: Histological and functional characterization of a mid-cervical contusion injury in rats

Authors: ***S. RANA**[1], H. M. GRANSEE[2], S. CORREA-CARDONA[2], M. PAREJA-CAJIAO[2], W. Z. ZHAN[2], G. C. SIECK[2,3], C. B. MANTILLA[2,3];
[1]Neurobio. of Dis., [2]Departments of Physiol. & Biomed. Engin., [3]Anesthesiol., Mayo Clin., Rochester, MN

Abstract: Damage to the spinal cord is a coalesced result of the primary insult to the cord and the secondary injury cascade that follows the impact. In order to identify therapeutic targets for spinal cord injuries (SCI), we must be able to use an injury model that closely represents a clinical setting. Mid-cervical contusion injury is one of the most common types of SCI and can lead to respiratory impairment via complete or partial paralysis of the diaphragm muscle. Thus, it is important to characterize the injury and extent of loss in the phrenic motor neuron (PhMN) pool that compromise respiratory function. In this study, we assessed the degree of damage following a 100kD unilateral C4 contusion injury in rats. We hypothesized that PhMN loss, cystic cavity formation and tissue scarring, as measured by chondroitin sulfate proteoglycans (CSPGs) expression, should increase progressively past 3 days post-injury (D) and stabilize by 7D and 14D. Intra-pleural injections of Alexa 488-conjugated cholera toxin subunit B were employed to label the PhMNs in the spinal cord. Immunohistochemical analysis by Wisteria Floribunda Agglutinin that labels perineuronal net formation was used to assess the extent of tissue scarring at the injury site. Quantitative confocal microscopy was used to compute volume of the cavity formed following injury and to compare loss of PhMNs ipsilateral and contralateral to the injury. Functional assessment of the injury was performed by obtaining diaphragm EMG recordings for ventilatory behaviors (eupnea, hypoxia-hypercapnia (10% O2 and 5% CO2)) and non-ventilatory behaviors (sustained airway occlusion, sighs, and sneezing). Our results show

that there is no difference between cyst volume at 3D, 7D, and 14D. There is progressive PhMN loss between 3D to 7D, and PhMN counts are comparable at 7D and 14D. Our results, thus, reveal that PhMN loss and tissue scarring progress past 3D to 7D, whereas cyst formation has plateaued by 3D. Ventilatory behaviors are not impacted by unilateral contusion, with no difference between ipsilateral and contralateral sides. Hence, assessment of higher force behaviors (e.g., coughing, sneezing) is imperative. Collectively, these findings illustrate the loss in tissue integrity and formation of an inhibitory environment at the injury site post contusion injury. These results also corroborate that a C4 unilateral contusion model is a useful tool in the assessment of effective therapeutic strategies for SCI, although, an earlier intervention might be needed to assess effectiveness on PhMN loss.

Disclosures: S. Rana: None. **H.M. Gransee:** None. **S. Correa-Cardona:** None. **M. Pareja-Cajiao:** None. **W.Z. Zhan:** None. **G.C. Sieck:** None. **C.B. Mantilla:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.10/U42

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant R01-HL096750

Title: Assessment of diaphragm EMG activity recovery following upper cervical spinal cord injury

Authors: *O. U. KHURRAM[1], Y. B. SEVEN[2], R. M. S. VASDEV[2], G. C. SIECK[2,3], C. B. MANTILLA[2,3];
[2]Physiol. and Biomed. Engin., [3]Anesthiology, [1]Mayo Clin. Col. of Med., Rochester, MN

Abstract: Motor units are normally recruited in an orderly manner in order to accomplish a range of behaviors. Across a range of ventilatory and non-ventilatory behaviors, the duration of non-stationarity in electromyographic (EMG) activity of the diaphragm muscle (DIAm) provides an estimate of the period of motor unit recruitment. Motor unit recruitment reflects central drive to the DIAm, which can be estimated by the root-mean-squared (RMS) EMG value 75 ms after the EMG burst onset (RMS75) in rats. Following unilateral DIAm paralysis induced by upper cervical spinal cord hemisection at C2 (SH), increased activity of the contralateral (uninjured) DIAm is sufficient to maintain ventilation. Over time following SH, there is spontaneous recovery of ipsilateral DIAm EMG activity, which may mitigate the compensatory increase in contralateral activity. We hypothesized that contralateral DIAm EMG measures such as duration of non-stationarity and RMS75 can quantitatively inform about recovery of ipsilateral DIAm activity following upper cervical spinal cord injury. Using chronic DIAm EMG recordings,

recovery of ipsilateral DIAm activity was observed in a subset of animals by 14 days post-SH (D14). However, peak DIAm RMS EMG activity was reduced compared to the pre-injury baseline in all cases. The contralateral DIAm displayed increased peak RMS EMG activity post-SH but there was no change in the duration of non-stationarity whether animals displayed recovery of ipsilateral EMG activity or not. By D14, contralateral DIAm RMS75 values were reduced in rats displaying recovery of ipsilateral DIAm activity compared to rats that did not. These results suggest that the period of DIAm motor unit recruitment (estimated by the duration of non-stationarity) during ventilatory behaviors did not change with SH or ipsilateral recovery. Central drive (contralateral DIAm RMS75) increased post-SH and returned to baseline in animals displaying recovery, and thus may be used to evaluate the extent of recovery of ipsilateral DIAm activity following spinal cord injury.

Disclosures: O.U. Khurram: None. **Y.B. Seven:** None. **R.M.S. Vasdev:** None. **G.C. Sieck:** None. **C.B. Mantilla:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.11/V1

Topic: D.10. Spinal Cord Injury and Plasticity

Support: fellowship from CONACyT No. 237231

Title: Minimally invasive approach to medular hemisection in rats. Technical advance

Authors: *F. A. ROCHA, R. RAMOS ZUÑIGA, G. GUDIÑO CABRERA;
U De G, Guadalajara, Mexico

Abstract: Spinal cord injury (SCI) interrupts the nerve connections between the brain and the rest of the body, and results in paralysis and loss of sensation below the level of injury. In the model of spinal cord hemisection in rats, it is necessary to have good access to the site that will be injured. Indeed, it is required to make a laminectomy in two vertebras at the level where the spinal cord injury will be. With this approach, a biomechanical destabilization occurs in the backbone of the animal, done by the removal of bone and supraspinous and interspinous ligaments. In the present work; we analyzed an approach that would maintain intact the spinous process and all the ligaments in the injury area, the main objective is to produce a minimum biomechanical injury. Males wistar rats were supplied by animal house of Centro de Investigaciones Biomedicas de Occidene (CIBO) of Instituto Mexicano de Seguro Social (IMSS), and mantainted in our animal house, with constant temperature (22 ± 2 °C) and 30 to 40% of relative humidity. The animals were maintained in acrylic cages, with rodent laboratory chow (PMI Nutrition International LLC, EUA) and tap water ad-libitum. All surgeries were done

under stereo microscope (ecleris, OM100f1) in sterile surgical room and with sterile surgical equipment. The laminectomy was done at the level T9 using an electric motor (Lynx, EM20K) with round dental drill. The spinal cord hemisection was performed with a homemade punch (silver, 3mm in diameter), in order to assure the lesion reproducibility and also, extract a portion of spinal cord. We analyzed the anatomy in-vivo with a computer axial tomography, and the locomotion with BBB scale. Our result shows a favorable recovery in 3 days, and observing minimal inflammation. With the computerized axial tomography we observed that the contralateral lamina, all the ligaments and the spinous process were preserved in the site of the injury and also were not being altered all the transverse process. In all spinal cord injury sites, we observed a cavity done with the punch. In conclusion, we designed a customized surgery and an hemisection device based on our requirement, satisfying all the parameters to withstand a standard animal model for spinal cord hemisection at the thoracic level without compromising the lesion reproducibility.

Disclosures: **F.A. Rocha:** None. **R. Ramos Zuñiga:** None. **G. Gudiño Cabrera:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.12/V2

Topic: D.10. Spinal Cord Injury and Plasticity

Title: The effect of combination therapy in the regeneration and locomotor recovery in rats with chronic spinal cord injury

Authors: *F. R. ADRIÁN, ESQ;
Proyecto Camina AC, México, Mexico

Abstract: The spinal cord injury (SCI) is a terrible condition that affect more than 250,000 people ever year around the world; the lesion produces an impairment not only of locomotor and sensitive capacities but also provokes deregulations of normal physiology. The acute phase is characterized by the activation of immunological response that progressively become permanent and continues in the chronic phase. The inflammatory response has an active participation in the formation of glial scar (GS), different researches had shown that GS exerts a negative effect on axon growth and stop the attempts made by the organism to reconnect neuronal pathways lost by the injury. A91 is a modified peptide that modulates the immunologic response when is applied after SCI, the effect is produced by the production of anti-inflammatory cytokines like IL-10, IL-4 and TGF β. This effect can be profited in a combinatory strategy directed to the restructuration of damage tissue and for consequence this could be used as alternative therapy to treat chronic SCI. The aim of this study is to evaluate the effect of a combinatory therapy in rats with chronic SCI and evaluate the locomotor recovery for 2 months and finally process the portion affected by

the injury and measure the numbers of axons positives to tyrosine hydroxylase and serotonin by an immunohistochemistry technic. For this purpose we evaluated the effect of five different combination therapies, the groups studied are: 1) Rats subjected to chronic SCI without further treatment; 2) Rats subjected to chronic SCI + scar removal; 3) Rats subjected to chronic SCI + scar removal + A91 immunization; 4) Rats subjected to chronic SCI + scar removal + A91 immunization + tissucol with mesenchimal cells; 5) Rats subjected to chronic + A91 immunization + tissucol with mesenchimal cells. The locomotor evaluation showed a higher recovery in the animals of group 5, this result is correlationed with the higher number of marked fibers with immunochemistry test. The results of this work demonstrate that a combinatory strategy in chronic SCI can profit positives the effects of different therapeutial approaches that had shown locomotor recovery and neuronal reconnection when are used as a unique treatment. Key words: A91, combination therapy, chronic spinal cord injury, locomotor recovery.

Disclosures: F.R. Adrián: None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.13/V3

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Kinematic analysis of disordered locomotion in common marmosets with spinal cord hemisection

Authors: *A. UCHIDA[1], T. KONDO[2], K. YOSHINO-SAITO[2], H. J. OKANO[5], M. NAKAMURA[3], H. OKANO[2], J. USHIBA[4];
[1]Grad. school of Fundamental Sci. and Technol., Keio Univ., Kanagawa, Japan; [2]Dept. of Physiol, [3]Dept. of Orthopaedic Surgery, Keio Univ., Tokyo, Japan; [4]Dept. of Biosci. and Informatics, Keio Univ., Kanagawa, Japan; [5]Sch. of Med., Jikei Univ., Tokyo, Japan

Abstract: Spinal cord injury (SCI) results in motor and sensory deficits below the level of the injury. Recently, some preclinical studies using non-human primates to establish treatments for recovery of impaired functions after SCI have been reported. These studies suggest the importance of combination of various therapeutic interventions such as stem cell transplantation and rehabilitations. However, conventional evaluation metrics of motor function after these interventions are lack in quantitativity and objectivity. In addition, few researches have focused on changes in the internal state of the neural system underlying behavioral changes after SCI. Information about the change in the internal state of the central nervous system (CNS) combined with reliable behavioral evaluations may greatly improve our understanding of the recovery process after SCI. Moreover, in order to enhance reacquisition of normal motor and sensory functions, we need to evaluate both performances and internal states of the CNS. In this study, to

establish new evaluation protocols for spontaneous recovery of locomotor function after hemisection SCI at C4/C5 level in common marmosets (N = 4), we developed the kinematic recording method and estimated the change in the internal state of the CNS by using principal component analysis (PCA) for the kinematic data. First, we established the motion capture system with 2 high speed cameras to record walking of common marmoset from both sides with sampling rate of 150 Hz. Sixteen reflective markers (3.97 mm diameter) were attached on 16 joints to track joint displacements in all-limbs. We analyzed the kinematic data in 4 time points before and after SCI. Horizontal shift between the shoulder and the metacarpophalangeal joints during walking was evaluated. At 2 weeks after the lesion, the forward horizontal shift was decreased and the backward shift was increased. However, at 12 weeks after the lesion, the horizontal shift recovered close to that before the lesion. Next, we estimated the internal state of the CNS in each time point by applying PCA for the data of temporal changes in 4 joint angles of paralyzed hindlimb in gait cycles. From the results of percent variance of principle components (PCs), the walking pattern before the lesion was coordinated such that PC1 and PC2 are weighted similarly. However, at 2 weeks after the lesion the pattern strongly depended on PC1. At 12 weeks after the lesion, the balance of PC1 and PC2 recovered close to the variance before the lesion. The current results suggest that our evaluation metric may provide novel insights for the recovery process of locomotor function after SCI, and contribute to future clinical researches on SCI.

Disclosures: **A. Uchida:** None. **T. Kondo:** None. **K. Yoshino-Saito:** None. **H.J. Okano:** None. **M. Nakamura:** None. **H. Okano:** None. **J. Ushiba:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.14/V4

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Helmsley Foundation Grant #2011PG-MED011

National Institute of General Medical Sciences Grant 8 P30 GM-103507

Ole A., Mabel Wise & Wilma Wise Nelson Research Chair Endowment

Title: Comparison of cervical cerebrospinal fluid flow between healthy participants and persons with spinal cord injury using cine velocity-mapping MRI

Authors: ***A. M. WILLHITE**[1], K.-J. JUNG[2], N. SETTIPALLE[1], B. WELLMAN[4], D. LORENZ[3], M. BOAKYE[1], S. J. HARKEMA[1];
[1]Neurolog. Surgery, [2]Radiology, [3]Bioinformatics & Biostatistics, Univ. of Louisville, Louisville, KY; [4]Radiology, Univ. of Louisville Hosp., Louisville, KY

Abstract: Purpose: The cerebrospinal fluid (CSF) flow in the cervical spine is expected to be reduced in persons with spinal cord injury (SCI) as a result of an increased resistance to the flow from stenosis in the subarachnoid space (SAS) and a reduced stroke volume of the heart. Therefore, the CSF flow in the SAS space at the cervical spine was compared between healthy volunteers and individuals with SCI using MRI. Methods: The flow velocity in the craniocaudal direction was acquired at the C4 spine level using a retrospectively gated phase-contrast cine sequence with a peripheral pulse taken from a finger and a velocity encoding range of 10 cm/s at 3 T MRI with head-neck and spine RF coils. The baseline velocity due to eddy current was corrected automatically using a specially designed algorithm which addressed the spatially dependent nature of eddy current. The region of detectable flow in SAS was segmented semi-automatically using a spectral analysis of the cine images of a complex data format. The flow in the segmented SAS was automatically analyzed for flow area, average peak velocities, average of standard deviations of systolic peak velocities, and average time to the velocity peaks using a customized analysis program. The extracted flow parameters were compared between 9 healthy volunteers and 9 participants with SCI. Mean values from healthy participants and SCI subjects were compared with a linear mixed effects model to account for repeated measurements from several subjects. Results:The heart rate was slower (877ms vs. 1094ms, $P<0.02$) and the time to the systole velocity peak was increased (70% vs. 82% of the cardiac cycle from the trigger, $P<0.11$) in SCI patients compared to healthy volunteers.. The flow area was reduced (122 mm2 vs. 108mm2, $P<0.23$) and both systolic (2.1 cm/s vs. 3.0 cm/s in the caudal direction, $P<0.03$) and diastolic (1.2 cm/s vs. 1.3 cm/s in the cranial direction, $P<0.09$) peak velocities were higher in participants with SCI than in healthy volunteers. In addition, the standard deviation of the systolic peak velocities in SAS was increased in SCI participants (2.1 cm/s vs. 3.0 cm/s, $P<0.03$). Conclusions: The flow velocity of SCI subjects was faster than that of healthy volunteers in both diastolic and systolic cardiac phases. This finding is contrary to the expected result of reduced flow velocity given that a slower heart rate and a reduced ejection fraction and stroke volume is often seen in SCI patients. Therefore, the reduced flow area may be a contributing factor to the increased velocity and the increased distribution of the systolic peak velocities in the flow area of the SAS.

Disclosures: **A.M. Willhite:** None. **K. Jung:** None. **N. Settipalle:** None. **B. Wellman:** None. **D. Lorenz:** None. **M. Boakye:** None. **S.J. Harkema:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.15/V5

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Neilsen Postdoctoral Fellowship Research Grant - 295998 (Kevin Hoy)

Neilsen Foundation- 221988 (Warren Alilain)

The MetroHealth System (Warren Alilain)

Title: Intravenous taxol therapy promotes breathing after cervical spinal cord injury

Authors: *K. C. HOY[1], F. J. JACONO[2,3], F. BRADKE[4], W. J. ALILAIN[5,6];
[1]Neurol., Metrohealth Med. Ctr., Cleveland, OH; [2]Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [3]Univ. Hosp. Case Med. Ctr., Cleveland, OH; [4]German Ctr. for Neurodegenterative Dis., Bonn, Germany; [5]MetroHealth Med. Ctr., Cleveland, OH; [6]Case Western Reserve Med. Sch., Cleveland, OH

Abstract: More than half of all spinal cord injuries (SCI) are at the cervical level. These injuries can result in the disruption of the respiratory motor pathways leading to the innervation of the diaphragm, resulting in the need for mechanical ventilation. To examine the neuronal circuitry involved in cervical SCI and potential interventions, our laboratory utilizes a C2 lateral hemisection. This model induces paralysis of the hemi-diaphragm ipsilateral to the hemisection. By leaving one side of the diaphragm still active, this model allows us to examine the breathing circuitry affected and spared by cervical SCI without the need for artificial ventilation of experimental subjects. A recent investigation has shown that the microtubule stabilizer paclitaxel (Taxol) can improve outcomes in thoracic injury. In the thoracic injury model, paclitaxel has been shown to reduce the perineuronal (PNN) net and increase serotonin (5-HT) after injury. However, its application in cervical injury is untested. Here we examined the therapeutic potential of paclitaxel on the recovery of breathing after a C2 spinal hemisection. Subjects received various (0.5mg, 0.25mg, & 0.125mg) doses of systemic i.v. paclitaxel at 48 hours post-injury. Preliminary data indicate that cervical SCI subjects that receive Taxol have improved respiratory motor function compared to vehicle treated animals in a dose dependent fashion. Based on these encouraging results investigating a combination treatment of paclitaxel and rehabilitative therapy is warranted. Future studies examining this multi-modality strategy are planned.

Disclosures: K.C. Hoy: None. **F.J. Jacono:** None. **F. Bradke:** None. **W.J. Alilain:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.16/V6

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Inoculation of neural antigens into the anterior chamber of the eye as a neuroprotective strategy after spinal cord injury in rats

Authors: *D. TOSCANO[1], B. PINEDA[2], A. IBARRA[2];
[1]Univ. Anahuac, Ciudad de Mexico, Mexico; [2]Univ. Anahuac, Mexico City, Mexico

Abstract: Objective Spinal cord injury (SCI) is considered a catastrophic disease due to the profound functional impairment and detriment in quality of life that it can cause to patients who suffer from it. Currently, there is no effective treatment for SCI. Inflammatory response is one of the main components of SCI pathophysiology as it is responsible for secondary damage. Anterior chamber associated immune deviation (ACAID) is a phenomenon that reduces the inflammatory response by producing tolerance to antigens inoculated in the anterior chamber of the eye. The aim of this study was to evaluate if the induction of ACAID with neural peptides represents a potential anti-inflammatory and neuroprotective strategy capable of improving motor function recovery after SCI in rats. Methods One-month-old Sprage Dawley rats were divided into two groups and inoculated in the anterior chamber of the eye with neural antigens (ACAID group) or PBS (control group). When rats reached the age of 2.5 months, moderate SCI was performed in both groups with the IH-0400 impactor at 200kdyn of force. Motor function and sensory recovery was assessed for 8 weeks with the BBB scale, Footprint test and von Frey Hair test, respectively. Results A statistically significant improvement in motor function recovery, as measured by the BBB scale, was observed between the ACAID group and controls ($p < 0.0001$). Also, a better performance in the footprint test was observed in the ACAID group. This was consistent with a higher number of motor neurons in the morphological analysis of the ACAID group compared to controls. As for sensory recovery, there was a tendency towards an increased improvement in the ACAID group. Conclusion This study supports immunomodulation achieved through anterior chamber associated immune deviation as a plausible neuroprotective strategy that could improve motor function recovery after traumatic SCI.

Disclosures: D. Toscano: None. **B. Pineda:** None. **A. Ibarra:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.17/V7

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Trans-spinal direct current stimulation changes the number and migration pattern of spinal cord adult-born cells

Authors: *Z. AHMED, S. SAMADDAR;
The Col. of Staten Island, Staten Island, NY

Abstract: Adult born cells (neurons or glia) in the nervous system are continuously produced throughout life. Recent studies implicated these adult born cells in learning (e.g. motor skill

learning). In the current study, we investigated the effects of trans-spinal direct current stimulation (tsDCS) on the number of adult born cells and their migratory pattern. Animals were implanted with tsDCS electrodes. Adult mice underwent tsDCS for five consecutive days and were simultaneously injected with 5'-bromo-deoxyuridine (BrdU). Cathodal tsDCS causes an increase of the number of BrdU-labeled cells to accumulate at the dorsal aspect of the spinal cord. Anodal tsDCS causes a shift of location of the BrdU-labeled cells toward the ventral aspect. In general, there was attraction of BrdU-labeled cells toward the cathode and repulsion from the anode. There was a significant increase in the number of labeled cells after anodal and cathodal stimulation. See figure (right). A, sham-control; B, cathode on the dorsum of the spinal cord; C, anode on the dorsum of the spinal cord. These reults could provide a novel mechanism of how tsDCS modulate function of the spinal cord. More importantly these results identfy a new approach to manipulate adult born cells that could be very useful in augmenting functional recovery after injuries.

Disclosures: Z. Ahmed: None. **S. Samaddar:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.18/V8

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NRF 2014M2C1B2048632

Title: The effects of hindlimb stretching and positioning exercise on locomotor function after spinal cord injury in rats

Authors: *E. SONG[1,2], H. JEON[1,2], S.-C. HAM[1,2], Y. KIM[3], Y. YOON[3], J. KIM[1,2];
[1]Korea Univ. Col. Hlth. Sci., Seoul, Korea, Republic of; [2]Rehabil. Sci. Program, Dept. of Publ. Hlth. Science, Grad. School,, Seoul, Korea, Republic of; [3]Korea Univ. Col. Med., Seoul, Korea, Republic of

Abstract: Rehabilitation exercise has been applied to prevent the progress of sensorimotor impairments in patients with spinal cord injury (SCI). In particular, most of all mainly have been focused in the effect of locomotion training. However, the effects of other rehabilitation approaches applied in patients with SCI except for locomotion training are still unclear and thus investigation for additional approaches is needed. Thus, we examined the effects of passive stretching exercise (SE) and positioning training (PT) on recovery of locomotor function. Spinal contusion was made in male Sprague-Dawley rats using NYU impactor on T12 spinal cord under anesthesia. Rats were randomly assigned to SE (n=10), PT (n=9), combined SE and PT (C, n=10) and no exercise (control, n=9). SE applied 6 muscles on hindlimb for 1 minute in each

motion alternately side repeated 2 sets. PT held quadripedal posture with plantar contact for 30 min. C performed 1 set of SE and 15 min of PT. These interventions were applied 5days/week for 4weeks. Locomotion recovery was assessed by BBB open field locomotor scale and combined behavior score (CBS). Hypersensitivity after SCI was evaluated by paw withdrawal threshold (PWT) with up - down method. Average, maximum speed and travelled distance that reflect the ability of physical activity were measured by using Panlab's smart tracking system. Luxor fast blue and cresyl violet staining was used to measure areas of cavities on epicenter. In the PT group, BBB was significantly increased at 10, 11, 12 and 13 days after SCI, and CBS also significantly decreased at 10, 11 and 12 days after SCI compared to control. BBB and CBS in the PT group slightly increased compared to them in the SE group during intervention. PWT in the PT group was significantly increased from 18 days to 28 days than it in the control. In all rehabilitation exercise groups, values of physical activities showed increased tendency on chronic stages and then only maximal velocity in the PT group were significantly faster than control group. Areas of epicenter cavity were no difference between each group. These results suggest that SE was no effect to locomotion recovery whereas PT with manually partial support may be more helpful than SE to recovery of locomotor function and hypersensitivity after SCI. This study suggests positive potential for therapeutic exercise to improve locomotor function in SCI rats, though the more work related with mechanism is still needed. This research was supported by the convergence technology development program for bionic arm through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT& Future Planning (2014M2C1B2048632).

Disclosures: **E. Song:** None. **H. Jeon:** None. **S. Ham:** None. **Y. Kim:** None. **Y. Yoon:** None. **J. Kim:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.19/V9

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Support for this study was provided by the Office of Research and Sponsored Programs at CMU, the College of Medicine, and the Field Neurosciences Institute and John G. Kulhavi Professorship.

Title: Mesenchymal stem cells that are genetically altered to overexpress SDF-1 increase growth of neural stem cell derived neurons and neural stem cell migration

Authors: *E. D. PETERSEN[1,2,3], A. N. STEWART[1,2,3], J. ROSSIGNOL[4,2,3], U. HOCHGESCHWENDER[4,3], G. DUNBAR[1,5,6,3];

[1]Central Michigan Univ., Mount Pleasant, MI; [2]Field Neurosciences Inst. Lab. for Restorative Neurol., Mount Pleasant, MI; [3]Program in Neurosci., Mount Pleasant, MI; [4]Col. of Medicine, Central Michigan Univ., Mount Pleasant, MI; [5]Dept. of Psychology, Mount Pleasant, MI; [6]Field Neurosciences Institute, 4677 Towne Ctr. rd. suite 101 Saginaw, MI, Saginaw, MI

Abstract: Neurodegenerative diseases are often characterized by a reduction in cell viability and density. The recruitment and integration of endogenous neural stem cells is a promising treatment strategy for the replacement of compromised cells in neurodegenerative disease. Stromal derived factor-1 (SDF-1) is a chemokine which functions as a signaling molecule, involved in differentiation, cellular locomotion, survival, and proliferation. Bone marrow derived mesenchymal stem cells were virally transfected to overexpress SDF-1 for treatment of spinal cord injury, with the goal of controlling cellular loss post-injury and inducing chemotaxic migration of endogenous neural stem cells to the lesion site. SDF-1 overexpressing MSCs were co-cultured with maturing stem cell derived neurons *in vitro* to determine the effects of secreted factors from SDF-1 overexpressing MSCs on process length, and electrical activity. Under-agarose chemo-attractant assays were performed to assess directional chemotaxis of multipotent neural stem cells towards the SDF-1 overexpressing stem cells. SDF-1 MSCs were determined to increase growth of neurons in co-culture models and induce directional chemotaxis superior to non-overexpressing MSC populations. MSCs, which overexpress modulatory chemokines, show potential for novel treatments targeting spinal cord injury and neurodegenerative diseases.

Disclosures: E.D. Petersen: None. **A.N. Stewart:** None. **J. Rossignol:** None. **U. Hochgeschwender:** None. **G. Dunbar:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.20/V10

Topic: D.10. Spinal Cord Injury and Plasticity

Support: CONACyT Grant #57204

Title: Combination therapy promotes neuroregeneration in rats after chronic spinal cord injury

Authors: *R. H. RODRIGUEZ BARRERA[1], A. IBARRA[2], A. FLORES-ROMERO[3], L. BLANCAS-ESPINOZA[5], K. SORIA-ZAVALA[3], E. GARCIA[7], Y. CRUZ[7], R. SILVA-GARCIA[6], V. BUZOIANU[8], A. FERNÁNDEZ-PRESAS[9], E. MENDIETA[4], M. KONIGSBERG[10], P. SUÁREZ-MEADE[2];
[1]Univ. Autónoma Metropolitana Iztapalapa, Mexico City, Mexico; [2]Neuroimmunology, Universidad Anahuac Mexico Norte, Df, Mexico; [4]Immunology, [3]Proyecto Camina A.C, Df, Mexico; [5]Immunology, [6]Inmunology, Hospital De Pediatría Cmn Siglo XXI, Df, Mexico;

[7]Neuroimmunology, Universidad Anáhuac México Norte, Df, Mexico; [8]Neurology, Hospital De Especialidades, Cmn Siglo Xxi, Df, Mexico; [9]Facultad De Medicina, Universidad Nacional Autonoma De Mexico, Df, Mexico; [10]Ciencias Biologicas, Universidad Autonoma Metropolitana Unidad Iztapalapa, Df, Mexico

Abstract: Numerous studies have focused on promoting axonal regeneration; however, only a few of them are directed to stimulate neural regeneration after a chronic spinal cord (SC) injury. Different laboratories have demonstrated the neuroregenerative effect of immunizing with neural-derived peptides (INDP). Additionally, evidence suggests that grafted mesenchymal stem cells (MSC) are able to stimulate neuroregeneration at the site of injury and its effect is potentiated when transplanted along with biological matrices, or with glial scar removal. These findings have led us to believe that combination therapies could result in higher neurological recovery. To the date, there is no information regarding the effect of INDP with other therapies. In the present study we combined INDP with scar removal and implantation of MSC impregnated in a biological matrix to evaluate neuroregeneration after chronic SC injury. This combination strategy was performed two months after SC injury. During the first set of experiments, we evaluated motor recovery and IL-4, IL-10, TNFα, IGF1, TGFβ, BDNF, NT3 and GAP43 gene expression in rats with SC contusion. After two months of injury, adult Sprague Dawley rats were allocated into 5 different groups: 1) Rats with no treatment; 2) Rats subjected only to scar removal; 3) Rats with scar removal and INDP; 4) Rats with no scar removal + INDP + fibrin glue impregnated with MSC; 5) Rats with scar removal + INDP + fibrin glue impregnated with MSC (n=12 per group). Results showed a significant improvement in motor recovery and higher expression of IGF1, TGFβ, IL-4, IL10, NT3, BNDF and GAP43 genes at the site of injury of rats treated with the combination therapy. In the the second experiment, rats were subjected to a complete SC transection. After two months of injury, animals were allocated into 2 groups: 1) Rats with no treatment; 2) Rats with scar removal + INDP + fibrin glue impregnated with MSC (n=6 per group). In this case, somatosensory evoked potentials (SSEP) and motor recovery were evaluated to demonstrate axonal regeneration. In the SSEP evaluation, comparison of latency and amplitude of the different components of the waves showed a significant improvement in rats treated with the combination therapy. Results suggest that this combination therapy could improve axonal regeneration at the site of injury and promote better motor recovery in rats with chronic SC injury.

Disclosures: R.H. Rodriguez Barrera: None. **A. Ibarra:** None. **A. Flores-romero:** None. **L. Blancas-espinoza:** None. **K. Soria-zavala:** None. **E. Garcia:** None. **Y. Cruz:** None. **R. Silva-garcia:** None. **V. Buzoianu:** None. **A. Fernández-presas:** None. **E. Mendieta:** None. **M. Konigsberg:** None. **P. Suárez-meade:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.21/V11

Topic: D.10. Spinal Cord Injury and Plasticity

Support: VA Pittsburgh Healthcare System Grant VA 02830

University of Pittsburgh PRO09120267

Title: Hand-related cortical sensorimotor activity after chronic tetraplegia

Authors: *D. A. ROYSTON[1,2,3], S. T. FOLDES[4,2,6], M. RANDAZZO[5], J. L. COLLINGER[4,6,2]; [2]Ctr. for the Neural Basis of Cognition, [3]Bioengineering, [4]Physical Med. and Rehabil., [5]Neurolog. Surgery, [1]Univ. of Pittsburgh, Pittsburgh, PA; [6]VA Pittsburgh Healthcare Syst., Pittsburgh, PA

Abstract: In spinal cord injury (SCI), somatic motor and sensory innervation is severely disrupted, which can substantially alter the function of related brain areas. Cortical activation changes during attempted and imagined movements have been observed in lower-limb paralysis, but the effects of tetraplegia on upper limb representations are still poorly understood. A deeper understanding of how this activity changes is needed to develop new rehabilitation strategies and would provide insight into the nature of neural plasticity. The objective of this study was to elucidate the effects of chronic tetraplegia on sensorimotor neural activity. We hypothesized that subjects with SCI would display decreased activation in sensorimotor-related areas during attempted hand movements compared to able-bodied controls, but increased activation during imagined movements. Fourteen able-bodied volunteers and eight participants with cervical SCI underwent functional magnetic resonance imaging (fMRI) to investigate cortical activity during attempted and imagined right hand grasps. We compared the peak activation between groups in several bilateral regions of interest (ROIs), including pre- and post-central gyri, supplementary motor areas (SMA), and posterior parietal cortex. Both subject groups showed significant task-related activity in all ROIs during both attempted and imagined grasps ($p<0.05$). Subjects with SCI showed lower activation during attempts than controls in contralateral pre- and post-central gyri ($p<0.01$), but greater activation in ipsilateral SMA and post-central gyrus ($p<0.05$). In contrast, subjects with SCI had greater cortical activation during imagery than controls ($p<0.05$) in bilateral pre- and post-central gyri and ipsilateral SMA and parietal cortex. Activity during attempts was much greater than that during imagery in contralateral pre- and post-central gyri ($p<0.001$) for controls, while this difference was not significant in subjects with SCI. Our findings show that individuals with chronic tetraplegia display less activity in cortical sensorimotor areas during attempted grasping and greater activity during imagined grasping compared to controls. Furthermore, control subjects showed much stronger peak activation in primary motor and sensory cortex during attempted movements than imagined movements, but the SCI group did not, possibly indicating a decrease in kinesthetic feedback during attempts or a decrease in motor inhibition during imagery. Continued investigation into the nature of these changes could aid in the development of better rehabilitation applications and neuroplasticity research.

Disclosures: **D.A. Royston:** None. **S.T. Foldes:** None. **M. Randazzo:** None. **J.L. Collinger:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.22/V12

Topic: D.10. Spinal Cord Injury and Plasticity

Support: BK21 Plus 10Z20130012372

Priority Research Centers Program 2009-0093829

Korea Health Technolgy R&D Project HI14C0522

Title: Effect of cerium nanoparticles on the neuronal regeneration following spinal cord injury

Authors: ***J. HONG**[1,2,3], J.-W. KIM[2,3], M. KIM[2,3], H.-W. KIM[2,3,4], J. HYUN[2,3,5];
[1]Dankook Univ., Cheonan, Korea, Republic of; [2]Dept. of Nanobiomedical Sci. and BK21 PLUS NBM Global Res. Ctr. for Regenerative Medicine, Dankook Univ., Cheonan, Korea, Republic of; [3]Inst. of Tissue Regeneration Engin. (ITREN), Dankook Univ., Cheonan, Korea, Republic of; [4]Dept. of Biomaterials Science, Col. of Dentistry, Dankook Univ., Cheonan, Korea, Republic of; [5]Dept. of Rehabil. Medicine, Col. of Medicine, Dankook Univ., Cheonan, Korea, Republic of

Abstract: Cerium nanoparticles (CeNPs) are known to reduce reactive oxygen species (ROS) and effective for the neuronal regeneration. ROS increment following spinal cord injury (SCI) arise secondary injury and finally lead to permanent functional dysfunction. In this study, we aim to delineate the effectiveness of CeNPs on the hydrogen peroxide-damaged cortical neurons and SCI rats for the first time, and to find the optimal dose of CeNP for neuronal regeneration *in vivo*. *In vitro* condition, we found that the optimal concentration of CeNPs (25-500μg/ml) increased survival rate of primary cultured cortical neurons following hydrogen peroxide application, and also decreased iNOS activity *in vitro*. Then CeNPs were applied into the injured spinal cord of rats with variable concentration (50μg-4mg/ml). We found that the cavity size and inflammatory cells were decreased at 1 and 8 weeks following SCI, and the locomotor function was improved more in CeNP-treated rats within the therapeutic range (250μg-1mg/ml) than in controls. The mRNA levels of iNOS and pro-inflammatory cytokines were decreased and interleukin-10 was increased at 1 week following CeNP application in a dose-dependent manner. We concluded that the application of the optimal concentration of CeNPs into injured spinal cord decrease iNOS and inflammation, and might be helpful to restore the locomotor function following SCI.

Disclosures: **J. Hong:** None. **J. Kim:** None. **M. Kim:** None. **H. Kim:** None. **J. Hyun:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.23/V13

Topic: D.10. Spinal Cord Injury and Plasticity

NIH U01EB15521,

NIH R01EB007615

Title: Electrophysiological mapping of rat sensorimotor lumbosacral spinal networks after complete paralysis

Authors: *H. ZHONG[1], P. GAD[2], R. ROY[2], J. CHOE[2], M. NANDRA[3], Y. TAI[3], Y. GERASIMENKO[2], V. EDGERTON[2];
[1]Integrative Biol. and Physiol., UCLA, Los Angeles, CA; [2]Univ. Of California Los Angeles, LOS ANGELES, CA; [3]Caltech, Pasadena, CA

Abstract: Stimulation of the spinal cord has been shown to have great potential for improving function after motor deficits caused by injury or pathological conditions. Using a wide range of animal models, many studies have shown that stimulation applied to the neural networks intrinsic to the spinal cord can result in a dramatic improvement of motor ability, even allowing an animal to step and stand after a complete spinal cord transection. Clinical use of this technology, however, has been slow to develop due to the invasive nature of the implantation procedures and the difficulty of ascertaining specific sites of stimulation that would provide optimal amelioration of the motor deficits. Moreover, the development of tools available to control precise stimulation chronically via biocompatible electrodes has been limited. Herein, we outline the use of novel technology in the spinal rat model, demonstrating the ability to identify and stimulate specific sites of the spinal cord to produce discrete motor behaviors in spinal rats using a multisite epidural array. The results demonstrate that spinal rats can stand and step when the spinal cord is stimulated tonically via epidural electrodes located at specific sites on the spinal cord. The quality of stepping and standing is dependent on the location of the electrodes on the spinal cord, the specific stimulation parameters, and the orientation of the cathode and anode during bipolar stimulation. Spinally motor evoked potentials (sMEP) produced during standing and stepping are critical tools to study selective activation of interneuronal circuits via responses of varying latencies. The present results provide evidence that the assessment of functional networks in the background of behaviorally relevant physiological states is likely to be a physiological tool of considerable importance in developing strategies to facilitate recovery of motor function after a number of neuromotor disorders.

Disclosures: **H. Zhong:** None. **P. Gad:** None. **R. Roy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

funds); Neurorecovery Technologies. **J. Choe:** None. **M. Nandra:** None. **Y. Tai:** None. **Y. Gerasimenko:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurorecovery Technologies. **V. Edgerton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurorecovery Technologies.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.24/V14

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant: R01 EB007615

Christopher and Dana Reeve Foundation

Kessler Foundation

Leona M. and Harry B. Helmsley Charitable Trust

NIH Grant: 5 P30 GM103507

Craig H. Neilsen Foundation

University of Louisville Foundation

Title: Characteristics of responses based on electrode and frequency selection during epidural stimulation in humans following SCI

Authors: *C. A. ANGELI[1,2], E. REJC[2], Y. CHEN[2], S. J. HARKEMA[2,1];
[1]Frazier Rehab Inst., Louisville, KY; [2]Univ. of Louisville, Louisville, KY

Abstract: Epidural stimulation of the lumbosacral spinal cord has been shown to facilitate standing and voluntary movement in individuals with complete spinal cord injury. Motor activity can be modulated in the presence of epidural stimulation by intent or sensory input. The location of the electrode array, selection of electrode combinations and stimulus frequency and intensity all influence muscle recruitment. In the present study we investigated the impact of selected stimulation configurations on motor evoked responses and generation of rhythmic motor patterns in the absence of volitional control. We compared configurations to previous work performed with a quad electrode and tested additional configurations with the increased flexibility allowed by the 5-6-5 electrode array. Stimulation was performed using a 5-6-5, 16-electrode array placed around L1-S1 spinal cord level in six individuals with motor complete injury. Stimulation frequencies ranged from 2- 60Hz with intensities ranging from 0.5V to 10.0V. Bipolar

stimulation was performed selecting 2 electrodes along the middle column of the array (27 mm longitudinal separation) to replicate previous work performed with a quad electrode. Bipolar stimulation was compared to wide field stimulations with a larger number of electrodes activated. Activation of lower extremity muscles varied according to location, intensity and frequency of stimulation. Higher voltages produced an oscillatory rhythmic pattern through a larger range of frequencies when compared to lower voltages. Configurations using a larger number of electrodes also produce greater motor activation and rhythmic responses when compared to bipolar stimulation. Detailed mapping of muscle recruitment and understanding responses through a wide frequency range is critical for optimization of stimulation configurations used in standing, stepping and voluntary activity.

Disclosures: **C.A. Angeli:** None. **E. Rejc:** None. **Y. Chen:** None. **S.J. Harkema:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.25/V15

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NSERC

CFI

Title: Recovery of locomotion is paralleled by time-dependent cortical plasticity after incomplete spinal cord injury

Authors: ***A. R. BROWN**, M. MARTINEZ;
Cell Biol. and Anat., Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada

Abstract: After an incomplete spinal cord injury (iSCI) at thoracic level, supraspinal structures, which initiate movement, and spinal circuits, which generate them, remain partially connected through residual descending pathways. In this scenario we and others have shown that, even after extensive iSCI, animals consistently recover voluntary quadrupedal locomotion spontaneously with minimal sensory inputs. What are the mechanisms of locomotor recovery and what structures contribute? Although the motor cortex is crucial for the execution of voluntary motor acts, its function over hindlimb locomotion after iSCI is poorly understood. In this study, we evaluated, for the first time, the cortical mechanisms of hindlimb locomotor recovery after iSCI in rats. Using intracortical microstimulation (ICMS) applied to the output layer V of the motor cortex to evoke movement, we examined the topographic arrangement of hindlimb movements within both motor cortices (motor maps) for a two-month period after iSCI (unilateral hemisection at T8). Locomotor performance was evaluated using well-established locomotor tasks. In intact rats, the location and size of the hindlimb motor maps was consistent between

animals, and maps contained representations of contralateral hindlimb joints exclusively. After iSCI, rats exhibited motor deficits on the side of the lesion that gradually recovered over the first five weeks. During this period, the hindlimb motor maps on the contralateral side (de-efferented cortex) disappeared, demonstrating that the contralateral motor cortex did not exert a control over the affected hindlimb. By contrast, the ipsilesional motor cortex dynamically reorganized over the same period to develop a novel representation of the ipsilateral hindlimb. The critical point here is that ICMS applied on the ipsilesional motor cortex evoked bilateral hindlimb movements, indicating that, after iSCI, the ipsilesional motor cortex functionally reorganizes to encode bilateral hindlimb movements. After the first five weeks of recovery, new plastic changes occurred within both motor cortices. The hindlimb motor maps within the ipsi- and contralateral motor cortex returned to normal showing that the motor cortices regain a normal control over the hindlimb. These data strongly suggest that cortical mechanisms actively participate in the re-establishment of a consistent bilateral locomotor pattern after iSCI. Importantly, these novel insights into the neural basis of recovery will help identifying therapeutic strategies to harness cortical activity and catalyze functional recovery following iSCI.

Disclosures: **A.R. Brown:** None. **M. Martinez:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.26/V16

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant DA031197

Title: Physiological correlates of depression in a rodent model of spinal cord injury

Authors: ***M. HOOK**[1,2], A. ACEVES[1], M. ACEVES[2];
[1]Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Inst. for Neurosci., College Station, TX

Abstract: Previously, we have shown that a subset of rats exhibit depression-like behaviors following spinal cord injury (SCI). These subjects display decreased sucrose preference, decreased open field activity and social exploration, and increased immobility on the forced swim test. These behaviors are thought to reflect symptoms of depression (i.e., decreased interest or ability to experience pleasure, psychomotor retardation, fatigue) that are analogous to those found in human patients. The current study extends these findings by examining the relationship between depression-like behavior and physiological function. Subjects were given a moderate contusion or sham injury and then assessed for depression-like behavior, on days 9-10, 19-20, and 29-30 post SCI, with a battery of established behavioral tests (sucrose preference, social

exploration, open field activity, burrowing activity, and appetite deviation). Using implantable telemetry devices, 24 hr recordings of home cage activity, body temperature, heart rate and heart rate variability were collected throughout the duration of the experiment. Locomotor performance (BBB scale) and measures of pain reactivity (girdle, tactile, and tail flick tests) were also assessed throughout the recovery period, to further examine the relationship between psychological and physical well-being. Using principal components and hierarchical cluster analyses, subjects were classified into two groups: exhibiting behaviors indicative of depression or no depression. The depressed and not-depressed groups displayed significant differences in both behavior and physiological function. Subjects characterized as depressed had significantly higher heart rates, and decreased heart rate variability, relative to intact and not-depressed SCI subjects. Body temperature and home cage activity did not differ between depressed and not-depressed SCI subjects, although both groups displayed lower activity levels than the intact controls. Replicating our previous study, therefore, we show that a subset of rodent subjects display signs of depression following SCI. Moreover in addition to behavior, the depressed and not-depressed subjects differ on measures of physiological function (e.g., decreased heart rate variability) that are also associated with depression in humans. These physiological differences further validate the rodent model of depression after SCI.

Disclosures: **M. Hook:** None. **A. Aceves:** None. **M. Aceves:** None.

Poster

337. Spinal Cord Injury I

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 337.27/V17

Topic: D.12. Kinematics and EMG

Support: Axion Research Foundation

Title: Computerized 3D gait analysis as a quantitative functional endpoint of spinal cord injury in African green monkeys

Authors: S. A. LIDDIE[1], R. J. GOODY[1], A. A. LEWIS[1], X. G. MORTON[1], D. FRANK[1], V. WOODS[1], M. STRUHARIK[1], *D. E. REDMOND, Jr[2], M. S. LAWRENCE[1];
[1]Res., RxGen, Inc., Hamden, CT; [2]Dept Psych & Neurosurg., Yale Univ., New Haven, CT

Abstract: The ability to assess quantitative functional recovery following musculoskeletal or neuromotor impairment or therapeutic intervention critically depends on establishing robust and reproducible means of assessing limb movement in the course of natural behaviors. We have applied computerized 3D gait analysis to evaluate hind limb gait parameters and joint angles to support longitudinal analysis of experimental models of spinal cord injury (SCI) in African green monkeys. A positive reinforcement training program was used to acclimate animals to treadmill

activity prior to data acquisition using a 4-camera analysis system (KinemaTracer™, Kissei Comtec, Japan). Mean and standard deviation for gait parameters (assessed from both hind limbs) recorded at 3 mph including cadence, stance duration, swing duration, percent time spent in double stance phase, stride length and step length were 199.8912.32 steps per minute; 0.360.03 sec; 0.250.02 sec; 0.060.02 sec; 46.369.33 cm and 25.112.7 cm, respectively. Characterizing baseline gait component measures, and associated data capture and analytic methods, has permitted our application of this test system to support various study designs including SCI efficacy studies. In addition to SCI, kinematic analysis also offers utility in other diseases that affect locomotion including stroke, osteoarthritic pain and Parkinson's, and combination of this approach with other quantitative measures such as plantar pressure analyses offers a powerful and highly translatable preclinical platform for evaluating motor deficits of the leg and associated recovery.

Disclosures: S.A. Liddie: None. **R.J. Goody:** None. **A.A. Lewis:** None. **X.G. Morton:** None. **D. Frank:** None. **V. Woods:** None. **M. Struharik:** None. **D.E. Redmond:** None. **M.S. Lawrence:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.01/V18

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Wings for Life

Djavad Mowafaghian Collaboration Grant

Canadian Institutes of Health Research (CIHR)

Title: Optogenetic and pharmacogenetic dissection of motor cortical plasticity following spinal cord injury in mouse

Authors: *B. J. HILTON[1,2], E. ANENBERG[3,4], T. C. HARRISON[3,4,5], J. D. BOYD[3,4], V. OHRI[2], T. H. MURPHY[3,4], W. TETZLAFF[1,2];
[1]Zoology, [2]Intl. Collaboration on Repair Discoveries (ICORD), [3]Psychiatry, [4]Brain Res. Ctr., Univ. of British Columbia, Vancouver, BC, Canada; [5]Mol. and Cell Biol., Univ. of California Berkeley, Berkeley, CA

Abstract: Regeneration of lesioned fibers is limited in the adult mammalian CNS, but many individuals that sustain incomplete spinal cord injuries undergo spontaneous functional recovery. Cortical plasticity is one mechanism thought to underlie this recovery, but how the motor cortex responds to spinal cord injury longitudinally is largely unresolved. We applied optogenetic motor

mapping in Channelrhodopsin-2 expressing mice before and at multiple time points after a C3/C4 dorsal column lesion to map changes in motor cortical topography and output within the same animals following bilateral ablation of the dorsal corticospinal tract and dorsal column sensory afferents. We find that cortical motor maps of the limbs are greatly diminished in area and magnitude in the early stages of injury. However, by 2-3 weeks post-injury, pre-injury forelimb and hindlimb output, map area, and short latency to movement are re-established. Anterograde tracing of the motor cortex revealed spontaneous axonal outgrowth to the red nucleus in injured animals compared to sham operated animals, suggesting that the re-establishment of motor cortical output occurred through cortico-rubrospinal circuits in addition to corticospinal circuits. To investigate how this plasticity impacts motor behaviour, we performed pharmacogenetic inactivation of dorsolateral corticospinal fibers and of cortico-rubrospinal fibers in injured and uninjured animals and found so far that dorsolateral corticospinal silencing led to the re-appearance of deficits in a ladder beam task that occurred early after injury. Optogenetic motor mapping provides a quantitative assessment of motor cortical output, can be performed repeatedly in individual animals from acute to chronic stages of injury, and represents a novel longitudinal quantitative assay to assess treatment following SCI.

Disclosures: B.J. Hilton: None. **E. Anenberg:** None. **T.C. Harrison:** None. **J.D. Boyd:** None. **V. Ohri:** None. **T.H. Murphy:** None. **W. Tetzlaff:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.02/V19

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Mission Connect

Neilsen Foundation

Title: Nociceptive stimulation increases blood brain barrier permeability following spinal cord injury

Authors: M. K. BRUMLEY[1], J. A. REYNOLDS[1], J. D. TURTLE[1], Y.-J. HUANG[1], S. M. GARRAWAY[3], *J. W. GRAU[2];
[1]Texas A&M Univ., College Station, TX; [2]Dept Psychol, Texas A&M Univ., College Sta, TX; [3]Emory Univ., Atlanta, GA

Abstract: Following spinal cord injury (SCI), noxious input disrupts normal physiology around the injury site and dramatically affects the recovery process. Noxious input provided by uncontrollable electrical stimulation increases cell death, promotes inflammation, impairs

locomotor function, and induces neuropathic pain (Garraway, 2014, Pain, 155: 2344; Grau, 2004, J Neurotrauma, 21: 1795). Others have shown that peripheral nerve injury induces an alteration within the spinal cord that increases blood-brain barrier (BBB) permeability through an inflammatory signaling pathway (Beggs, 2010, Mol Pain, 6: 74; Echeverry, 2011, J Neurosci, 31: 10819). Here, we explored how noxious stimulation affects BBB permeability after a spinal contusion injury. Subjects received a moderate spinal contusion injury at T12 using the MASCIS device. Twenty-four hours later, six minutes of intermittent electrical stimulation was applied to the tail and tissue was collected from the lesion site 1 hour, 3 hours, and 24 hours later. Full spectral analysis of protein isolates showed absorbance peaks at 420nm in subjects that received noxious input, indicating an increased concentration of hemoglobin. To confirm these results, immunoblotting of the protein isolates was performed. Noxious input induced increased concentrations of hemoglobin alpha in the lesion site. We propose that increased levels of hemoglobin alpha indicate increased permeability of the BBB, which allows for increased passage of cells into the damaged tissue. Therefore, our data suggest that the detrimental effects of noxious input following SCI may involve aberrant BBB permeability. Preliminary data suggest that the increase in BBB permeability is linked to the activation of cell death pathways and purinergic signaling. Future work is examining additional markers directly related to BBB permeability as well as the trafficking of other cell types across the BBB. [Supported by Neilson Foundation and Mission Connect grants to JG]

Disclosures: M.K. Brumley: None. **J.A. Reynolds:** None. **J.D. Turtle:** None. **Y. Huang:** None. **S.M. Garraway:** None. **J.W. Grau:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.03/V20

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Canadian Institute of Health Research

Multiple Sclerosis Society of Canada

Title: PDGFRα-positive progenitor cells form myelinating oligodendrocytes and Schwann cells following contusion spinal cord injury

Authors: *P. L. ASSINCK[1], G. DUNCAN[1], J. PLEMEL[2], M. LEE[1], J. LIU[1], D. BERGLES[3], W. TETZLAFF[1];
[1]ICORD/University of British Columbia, Vancouver, BC, Canada; [2]Hotchkiss Brain Institute,, Univ. of Calgary, Calgary, AB, Canada; [3]Johns Hopkins Univ. of Med., Baltimore, MD

Abstract: Contusive spinal cord injury (SCI) results in considerable demyelination of spared axons, which impairs signal transduction and may leave axons vulnerable to degeneration. Both oligodendrocytes (OL)s and Schwann cells remyelinate denuded axons in the subsequent weeks and months following SCI. NG2 cells, characterized by the near ubiquitous co-expression of platelet derived growth factor receptor α (PDGFRα) in the uninjured central nervous system (CNS), are oligodendrocyte progenitors (OP)s which may serve as a source of new OLs following SCI. PDGFRα-CreERT mice were crossed with Rosa26-YFP mice and administered tamoxifen to label OPs two weeks prior to contusive thoracic spinal cord injury. In the uninjured spinal cord we found that YFP was expressed in NG2+ OPs at very high efficiency, as well as vascular associated cells (pericytes) and fibronectin+ fibrocytic cells in the spinal roots. Following injury, many recombined cells continue to express the PDGFRα+, Olig2 and NG2, indicative they have remained as OPs, but substantial differentiation into new oligodendrocytes (CC1+) was observed, responsible for de novo ensheathment of >30% of the myelinated axons by three months. Strikingly, the majority of P0+ Schwann cells in the spinal cord expressed YFP, suggesting they originated from central nervous system PDGFRα+ OPs. Furthermore, analysis of Olig2-CreERT:Rosa26-YFP mice and P0-CreERT:Rosa26-YFP mice suggests that after injury, Schwann cells are derived from both a centrally derived Olig2 positive population and a peripherally derived P0 positive population (nerve roots or meninges). Overall, this work reveals phenotypic plasticity of PDGFRα precursors following spinal cord injury as a source of the new remyelinating Schwann cells and oligodendrocytes in the injured spinal cord.

Disclosures: **P.L. Assinck:** None. **G. Duncan:** None. **J. Plemel:** None. **M. Lee:** None. **J. Liu:** None. **D. Bergles:** None. **W. Tetzlaff:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.04/V21

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant R25GM060507

Title: Significant gene expression and localization of fatty acid binding protein 4 following spinal cord injury in rats

Authors: ***J. C. LICERO CAMPBELL**, M. SERRANO ILLÁN, K. CORDERO CABAN, A. DURAN, J. FIGUEROA, M. DE LEON;
Loma Linda Univ., Loma Linda, CA

Abstract: The pathology of traumatic spinal cord injury (SCI) results from both the initial mechanical insult and the secondary processes that occur over hours and days following injury.

This secondary insult represents an important window for intervention and is characterized by a marked deregulation in lipid metabolism leading to inflammation. The present study investigates the expression and roles of fatty acid-binding protein 4 (FABP4) in rats post spinal cord injury. The multicenter animal spinal cord injury study (MASCIS) injury model was used to generate a contusion to the T-10 spinal segment of rats. Spinal cord samples were collected at 1, 3, 7, 14, and 28 days post-injury and analyzed to determine the spatiotemporal expression of FABP4 using immunohistochemistry and real-time RT-PCR. Here, we show that injury to the spinal cord results in a dramatic up- regulation in the mRNA and protein levels of FABP4. Notably, this expression was most prevalent in bone marrow derived M1 macrophages, and microglia. To investigate the potential role of this protein in functional recovery after SCI, the rats received intrathecal administration of the FABP4 inhibitor, BMS 309403. We show that animals receiving the FABP4 inhibitor exhibited improved locomotion after injury when compared to vehicle treated rats. Interestingly, this beneficial effect was not associated with the regulation of pro-inflammatory cytokines mRNA levels in the injured spinal cord. Altogether, our findings are the first to show a robust increase in protein and mRNA levels of FABP4 following SCI and a possible functional role in this context. These data suggest that FABP4 may play a major role in hyper- acute inflammatory responses and functional recovery after injury, representing an attractive target for intervention.

Disclosures: **J.C. Licero Campbell:** None. **M. Serrano Illán:** None. **K. Cordero Caban:** None. **A. Duran:** None. **J. Figueroa:** None. **M. De Leon:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.05/V22

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Shriner's Hospitals for Children Grant #86000

Title: Influence of cervical propriospinal interneurons in recovery of forelimb function after spinal cord injury

Authors: *I. S. SHEIKH, Y. LIU, K. KEEFE, X. TANG, G. SMITH;
Neurosci., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Spinal cord injury (SCI) effects approximately 100,000 people/year resulting sensory and motor paralysis below the level of injury. SCI causes demyelination, degeneration of axon tracts and neuronal death at the site of injury. Attempts to improve axon regeneration from supraspinal centers have proven difficult due to the long distances required, presence of inhibitory molecules and diminished regenerative program. Propriospinal interneurons (PNs) are

found throughout the spinal cord, forming simple and complex circuits between different spinal segments. PNs function by integrating supraspinal motor and sensory information to organize locomotion. Studies postulate that spared PNs can reorganize and contribute to functional recovery by establishing relays between lesioned axons and motor neurons. However, to what extent PNs contribute to recovery of forelimb reaching and grasping following SCI is not known in rodents. In our first study, we examined a novel two-viral vector technique for silencing cervical PNs in adult rats. The first is a retrograde transportable lentivirus expressing tetracycline-inducible tetanus toxin injected at PN synaptic terminals near motor neurons. The other is AAV expressing tet-On, which is injected at the PN cell bodies. No forelimb deficits were manifested post-injections in normal animals. Doxycycline administration produced no observable forelimb deficits in paw preference, grooming and grip-strength. Histological analysis showed neuronal GFP tagged tetanus toxin expression in the cervical spinal cord. In our second study, we tested recovery of forelimb motor function following a unilateral C5 hemisection. We observed partial recovery of forelimb deficits in the IBB forelimb test and in grip-strength 6 weeks post-lesion, which was lost upon doxycycline-induced silencing of C3/C4 PNs. No recovery was observed in paw-preference and grooming behaviors. These findings may indicate that PNs play a minor role in forelimb motor function in normal rats. However, after loss of direct supraspinal input, they may participate in recovery of forelimb function following a spinal cord injury. Further studies are required to determine the extent PNs are involved in forelimb recovery and the endogenous supraspinal connections onto PNs in the rat.

Disclosures: **I.S. Sheikh:** None. **Y. Liu:** None. **K. Keefe:** None. **X. Tang:** None. **G. Smith:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.06/V23

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation

advanced ERC grant (Nogorise)

Christopher and Dana Reeve Foundation

Title: Assessing the functional relevance of compensatory vs. regenerative sprouting of reticulospinal fibers after incomplete spinal cord injury using DREADD

Authors: ***A. ENGMANN**[1,2], M. SCHNEIDER[1], A. JESKE[1], N. RAPPO[1], R. SCHNEIDER[1], O. WEINMANN[1], H. KASPER[1], M. WIECKHORST[1], M. E. SCHWAB[1,2];

[1]Univ. Zürich, Brain Res. Inst., Zürich, Switzerland; [2]ETH Zurich, Hlth. Sci. and Technol., Zurich, Switzerland

Abstract: The brainstem has often been thought to be hardwired, and bulbospinal fiber growth after spinal cord injury has not been well studied. However, recent data suggest differently: Following axotomy after a spinal cord lateral hemisection in adult rats, the gigantocellular reticular nucleus (NRG), one of the main components of the reticular formation, has been shown to sprout vigorously rostral to the lesion site (regenerative sprouting). Some of these severed fibers re-connect to propriospinal neurons, which run down on the intact hemicord and re-cross (plastically) caudal to the lesion, thereby relaying bulbospinal commands to denervated target areas (Filli & Engmann et al., J Neuroscience 2014). Interestingly, the spared NRG axons on the contralesional side also show sprouting in the lumbar spinal cord, projecting branches across the midline into the denervated hemicord (compensatory sprouting, Zörner & Bachmann et al., Brain 2014). Animals and humans with this type of incomplete spinal cord injury have been described to show pronounced recovery of hindlimb function: Six to eight weeks after the lesion, overground locomotion of the hindlimbs nearly recovered back to baseline levels. In order to investigate the functional relevance of regenerative vs. compensatory NRG sprouting, we used a pharmacogenetic neuromodulative approach: We used two viral vectors (AAV-DIO-DREADD in the NRG and AAV-Cre in the spinal cord) which we injected in animals that had recovered from a unilateral spinal hemisection injury at the spinal level C4. This technique allowed us to introduce the inactivating DREADD receptor hM4Di in a projection-specific way either into the axotomized NRG neurons that had sprouted rostral to the lesion site (regenerative sprouting fibers), or into the intact NRG neurons that had sprouted over the midline in the lumbar spinal cord (compensatory sprouting). Detailed functional analyses of joint movements and limb kinetics during overground walking, wading and swimming under conditions where one or the other (or both) of these novel NRG projections are inactivated will lead to a more detailed understanding of the functional relevance of the different modes of plastic fiber tract rearrangements that occur spontaneously after a major but incomplete spinal cord injury.

Disclosures: A. Engmann: None. **M. Schneider:** None. **A. Jeske:** None. **N. Rappo:** None. **R. Schneider:** None. **O. Weinmann:** None. **H. Kasper:** None. **M. Wieckhorst:** None. **M.E. Schwab:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.07/V24

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NINDS 1R01NS076976a

Title: Olfactory ensheathing cells reduce inhibitory factors at the astroglial scar-border after a complete mid-thoracic spinal cord transection

Authors: R. R. KHANKAN[1,2], K. L. INGRAHAM[1,2], J. R. HAGGERTY-SKEANS[1], K. G. GRIFFIS[1,2], P. K. MANN[1], H. ZHONG[1], R. R. ROY[1,3], V. R. EDGERTON[1,3], *P. E. PHELPS[1,3];
[1]Integrative Biol. and Physiol., [2]Molecular, Cell. & Integrative Physiol. IDP, UCLA, Terasaki Life Sci. Building, Los Angeles, CA; [3]Brain Res. Inst., UCLA, Los Angeles, CA

Abstract: After spinal cord injury, astrocytes and meningeal fibroblasts up-regulate and secrete chondroitin sulfate proteoglycans (CSPGs) that contribute to the inhibitory environment formed at the lesion site. We asked if CSPGs were reduced in olfactory ensheathing cell (OEC) and skin fibroblast (FB, control) transplanted spinal cords after a complete T8-9 transection. We quantified anti-CS-56 luminance at the astroglial border and within the lesion core of every 16th spinal cord section for each spinal rat. CS-56-immunoreactivity was lower in OEC compared to FB-treated rats in both rostral and caudal GFAP-positive scar borders and in the lesion core at 2 and 4 weeks post-injury. We evaluated the presence of inhibitory myelin after injury by staining for Oil Red O, a marker of myelin debris. OEC-treated spinal cords had lower levels of myelin debris than those treated with FBs at 2 weeks post-injury. Oligodendrocyte progenitor cells (OPCs) also can contribute to the inhibitory scar formed after a spinal cord injury, and therefore we examined the number of OPCs at the scar border with glutathione-S transferase-pi (GST-pi) immunolabeling. GST-pi is uniquely expressed by OPCs and oligodendrocytes in the CNS: however, the localization of GST-pi in the nucleus is transient in early oligodendrocyte progenitors and then translocates to the cytoplasm during maturation. We quantified cells with distinct nuclear GST-pi and found that at both 2 and 8 weeks, fewer cells with nuclear GST-pi were found in OEC compared to FB-treated rats at both the rostral and caudal borders. Collectively, these data imply that OECs can modulate the presence of inhibitory factors at the lesion site after a spinal cord injury. Previously we reported that both OECs and FBs migrate widely into the lesion core 1 week after transplantation. By 2 weeks both cell transplants are eliminated from the lesion core and remain only in the rostral and caudal stumps. While OECs survive longer than FBs, neither cell type survived beyond 8 weeks. Our data suggest that the prolonged presence of OECs may further promote a beneficial tissue healing response. To enhance cell survival we administered cyclosporine-A (CSA) to suppress immune-mediated rejection and then compared how OECs and FBs modify inhibitory molecules associated with the injury site at 2 and 8 weeks post-lesion. CSA enhanced the survival of both OECs and FBs at both ages as more OECs and FBs were found in the lesion core with than without CSA treatment. In some CSA-treated spinal cords OECs survived at 8 weeks and formed a bridge between the rostral and caudal stumps.

Disclosures: **R.R. Khankan:** None. **K.L. Ingraham:** None. **J.R. Haggerty-Skeans:** None. **K.G. Griffis:** None. **P.K. Mann:** None. **H. Zhong:** None. **R.R. Roy:** None. **V.R. Edgerton:** None. **P.E. Phelps:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.08/V25

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Wings For Life Grant WFL-CA-005/15

Title: Systemic LPS increase rehabilitative training efficacy after cervical chronic spinal cord injury in rats

Authors: *A. TORRES ESPÍN, A. LING, J. FORERO, K. K. FENRICH, K. FOUAD; Physical Therapy, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Unfortunately, there are still no treatments to repair the injured spinal cord. However, in the clinical setting, rehabilitative training is currently one of the most successful therapies to promote recovery. Such training augments functional recovery through various mechanisms referred to as neuroplasticity including strengthening of spared connections, neurite outgrowth, changes in cellular properties and fine-tuning of these rearranged networks. A significant challenge for rehabilitative training following spinal cord injury (SCI) is the timing of the training onset. Similar to findings after brain injuries, various studies have reported that starting training in the early stages (subacute) following injury is much more effective in promoting recovery than delaying training onset to the chronic phase, weeks after the trauma. However, considering that accidents causing SCI are often poly-traumatic events, rehabilitative training of motor tasks in the subacute phase is frequently not an option. Thus, finding approaches to increase the training efficacy during chronic SCI becomes mandatory. Here we investigated the increase of inflammation as a possible attempt for enhancing neuroplasticity and training efficacy after chronic SCI. Thus, we analyzed whether the systemic administration of lipopolysaccaride (LPS), that induced neuroinflammation, in the chronic SCI can improve the training efficacy in a specific task such as single pellet reaching. Three groups of animals were studied. One group of rats received training during 8 weeks starting 8 weeks after cervical SCI, another group of rats received the same training plus the LPS administration and a third group of rats received the LPS administration only. Our results demonstrated that rehabilitative training efficacy of arm/forelimb reaching during the chronic phase of SCI can be increased by the systemic application of LPS. Thus, we propose that neuroinflammation may play a role in the window of opportunity for rehabilitative training success after SCI. The modulation of this neuroinflammation can be a new approach to reopen the window for rehabilitative training even weeks after SCI.

Disclosures: A. Torres Espín: None. **A. Ling:** None. **J. Forero:** None. **K.K. Fenrich:** None. **K. Fouad:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.09/V26

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Kinematics analysis by the Frechet dissimilarity method in intact and spinal cord injury rats treated with tamoxifen and exercise

Authors: *L. P. OSUNA CARRASCO[1], J. R. LÓPEZ RUIZ[1], G. MENDIZABAL RUIZ[1], I. JIMÉNEZ ESTRADA[2], J. BAÑUELOS PINEDA[1], S. H. DUEÑAS JIMÉNEZ[1];
[1]Univ. of Guadalajara, Guadalajara, Mexico; [2]CINVESTAV-IPN, México, DF., Mexico

Abstract: Pharmacological treatments, and bipedal or quadrupedal treadmill training have been made in spinal cord injured rats. Although treadmill training has been recognized to improve locomotion and tamoxifen demonstrated it neuroprotective effects in the nervous system, the combined application shows better results in the movement integration of the hindlimbs. The purpose of this study is to quantify the kinematics parameters in intact rats and in the same animals 15 and 30 days after injured and with tamoxifen, exercise and tamoxifen plus exercise treatments. A penetrating injury was made in the T13-L1 spinal cord segment. Kinematics of hindilmbs was quantified by a software developed in our laboratory that uses Frechet dissimilarity algorithm, designed to qualify the normal trace of the angular joint and pendulum like movement behavior and comparing with the traces obtained from the strides reconstruction at 15 and 30 days post-injury in the same injured rats. At 30 days post-injury, the ipsilateral hindlimb pendular curves had a statistical significant reduced frechet dissimilarity than 15 days post-injury. This results are due mainly by exercise benefits. The Frechet dissimilarity was large in untreated rats. In the contralateral hindlimb pendular curves there was no statistical significance between 15 and 30 days post-injury rats. At 30 days post-injury, there was a statistical significant decrease in frechet dissimilarity occurred in contralateral knee and ankle joint values as compared with 15 days post-injury in the exercise treated rats. A qualitative, hematoxilin and eosin histological evaluation of the spinal cord indicates a regenerative process in rostral and caudal to the injury level. The tissue was better preserved in tamoxifen, exercise or combined treatments. Our results favored a combinatorial treatment for repairing spinal cord penetrating injury.

Disclosures: **L.P. Osuna Carrasco:** None. **J.R. López Ruiz:** None. **G. Mendizabal Ruiz:** A. Employment/Salary (full or part-time):; University of Guadalajara. **I. Jiménez Estrada:** A. Employment/Salary (full or part-time):; CINVESTAV-IPN. **J. Bañuelos Pineda:** A. Employment/Salary (full or part-time):; University of Guadalajara. **S.H. Dueñas Jiménez:** A. Employment/Salary (full or part-time):; University of Guadalajara.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.10/V27

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation

advanced ERC grant (Nogorise)

ETH 'Eat to learn to move' network

Christopher and Dana Reeve Foundation

Title: Sprouting and 'side-switch' of contralateral corticospinal fibers in the spinal cord after large unilateral cortical stroke

Authors: *J. KAISER[1,2], A.-S. WAHL[1], M. SCHWAB[1,2];
[1]Brain Res. Inst., Zürich, Switzerland; [2]Dept. Hlth. Sci. and Technol., ETH, Zurich, Switzerland

Abstract: Following a large unilateral stroke of the sensory-motor cortex, the contralateral, spared cortex was shown to be activated for restoring a certain low level of function. On the spinal level, the remaining, intact corticospinal tract forms newly outsprouting fibers within the cervical enlargement, a process that is causally related to the functional recovery of the forelimb. Little is known about the time course, the anatomical details and the underlying molecular mechanisms of this sprouting process. Adult mice are subjected to a unilateral photothrombotic stroke of the right primary as well as pre-motor cortex ablating >90% of the descending projections of the corticospinal tract. The corticospinal tract of the contralesional motor cortex is labeled using an anterograde tracer to label spinal projections in healthy as well as stroked C57/Bl6 wild type mice. In comparison, the corticospinal fibers of mutant animals, in which the growth inhibitory protein Nogo-A protein was knocked out conditionally or conventionally, are studied to assess the sprouting profile in a more growth permissive environment. All animals are analyzed for their cervical sprouting, branching and midline crossing profile at different time points after the thrombotic insult. This analysis will focus on the three possible types of fibers descending from the contralesional cortex that could play a key role in functional recovery: ipsilateral projections, pre-existing (re-)crossing projections as well as newly forming midline crossing fibers. Additionally, using transgenic reporter mouse lines, different cell types are also characterized for their potential involvement in this sprouting/branching process.

Disclosures: J. Kaiser: None. **A. Wahl:** None. **M. Schwab:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.11/V28

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Treadmill training reduces mechanical allodynia and thermal hyperalgesia after chronic mouse spinal cord contusion injury

Authors: *C. SLIWINSKI, T. NEES, M. MOTSCH, N. WEIDNER, A. BLESCH;
Spinal Cord Injury Ctr. Univ. Hosp. Heidelberg, Heidelberg Univ., Heidelberg, Germany

Abstract: A large proportion of patients suffering from spinal cord injury (SCI) develop chronic neuropathic pain (CNP). Despite its frequently severe and disabling nature, pharmacologically effective treatment options are still lacking and a causal relationship between aberrant structural remodeling and the development of CNP has not been clearly established. Previously, we and others have shown that sensorimotor training early after SCI can ameliorate mechanical allodynia but not thermal hyperalgesia. To determine whether similar training paradigms are effective in the subchronic / chronic phase of SCI, correlates of below level neuropathic pain including mechanical and thermal abnormalities were analyzed in the hindpaws between 35 and 70 days after a moderate T11 spinal cord contusion injury (50 kDyn) in female C57BL/6 mice. Treadmill training was initiated 6 weeks post-injury to investigate the effect of moderate physical therapy on pain behavior and associated morphological changes. Untrained SCI mice showed mechanical allodynia measured with small-diameter von Frey filaments (0.16g) at 35 days post-injury (dpi), which was stable until the end of the experiment (ANOVA $p<0.05$; PLSD $p<0.05$ at 70dpi). Interestingly, when tested for normally noxious stimuli using stronger filaments (0.6g; 1.4g), SCI mice showed a significant hyporesponsiveness compared to sham animals (ANOVA $p<0.001$, PLSD $p<0.0001$ at 70dpi). In addition, SCI animals showed a significant decrease in the response latency to heat stimuli at 35 dpi compared to sham mice, which remained stable (ANOVA $p<0.001$ at 70dpi). Moderate treadmill training was performed 2 x 15 minutes/day, 5 days/week over 5 weeks increasing the velocity of the treadmill from 0.15 m/s to 0.26 m/s to adapt to the recovery of SCI mice. Treadmill training positively influenced both mechanical allodynia and thermal hyperalgesia. The response rate to light mechanical stimuli (0.16g) was significantly reduced in trained SCI mice compared to untrained SCI mice starting at 4 weeks of training (ANOVA $p<0.05$; PLSD $p<0.05$). A significant reduction in thermal hyperalgesia was evident as early as 2 weeks after initiation of training (55 dpi) in the SCI group and remained stable until the end of the experiment (ANOVA $p<0.001$; PLSD $p<0.05$). Current data further suggest a training-mediated normalization in the thermal place preference test (22°C vs. 17°C) with trained SCI spending similar times on the 22°C side as sham animals. Potential mechanisms underlying the observed pain-related behavior including sprouting of peptidergic nociceptive fibers and changes in inhibitory interneurons in the dorsal spinal cord are currently analyzed.

Disclosures: **C. Sliwinski:** None. **T. Nees:** None. **M. Motsch:** None. **N. Weidner:** None. **A. Blesch:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.12/V29

Topic: D.10. Spinal Cord Injury and Plasticity

Support: ISRT NRB 107

MRC Grant MR/K022911/1

Royal Society RG2010/R2

Title: Kinematics analysis of locomotor function following treatment with epidural stimulation, locomotor training and intraspinal chondroitinase-ABC in a severe contusion injury

Authors: *R. M. ICHIYAMA[1], Y. D. AL'JOBOORI[1], C. C. SMITH[1], K. O. CHEN[1], S. CHAKRABARTY[1], J. W. FAWCETT[2], E. M. MUIR[3];
[1]Univ. Leeds, Leeds, United Kingdom; [2]Clin. Neuroscience, Brain Repair Ctr., [3]Physiology, Develop. & Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Electrical epidural stimulation (ES) of the lumbar spinal cord (L2 to S1) has previously been shown to improve locomotor function in complete transection models of rat spinal cord injury in conjunction with monoaminergic and serotonergic agonists and bipedal locomotor training; however, this functional improvement has only been observed under training conditions (i.e., bipedally, body weight supported, under ES) and previous evidence has shown that rehabilitation up-regulates chondroitin sulphate proteoglycan in the lumbar spinal cord therefore likely restricting synaptic plasticity. We hypothesized that adding lentiviral chondroitinase (LV-ChABC) locally after injury would facilitate synaptic plasticity and thus allow for enhanced functional recovery. Here we demonstrate that the use of ES (40 Hz; L2) and locomotor training following severe spinal contusion injury (T9/10) leads to improved locomotor function in both saline and LV-ChABC groups. Adult Sprague-Dawley rats received a severe spinal contusion injury (T9/10), epidural electrode implantation at segmental levels L2 and S1 and intra-spinal injections of LV-ChABC or saline (control). Rats were then randomly assigned to one of four groups: cage control, training only, ES only or ES+training. Rats in either trained group stepped bipedally-quadrupedally on a body weight supported treadmill (5-16 cm/s) (5 days/week, 20 mins/day) for 8 weeks. By the end of the 8-week period rats in the Saline/LV-ChABC+ES+training group showed improvements not only in supported treadmill stepping ability but also in open field locomotion (BBB), with combination saline/LV-ChABC treated animals achieving the highest overall increase in mean BBB score compared to Saline/LV-Chase

controls. Kinematics analysis revealed more detailed differences in stepping characteristics and pattern following 8 weeks of training. Rats in the LV-ChABC+ES+training group showed greatest coordination and consistency in stepping patterns compared to all other groups. Therefore these results suggest that a combination of step training and ES in an incomplete model of SCI successfully improved locomotor function further than either therapy administered alone with LV-ChABC+ES+training group improving the most overall; with animals not only improving in treadmill step performance but were also able to transfer this skill to an open field task.

Disclosures: R.M. Ichiyama: None. **Y.D. Al'joboori:** None. **C.C. Smith:** None. **K.O. Chen:** None. **S. Chakrabarty:** None. **J.W. Fawcett:** None. **E.M. Muir:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.13/V30

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Internationale Stiftung für Forschung in Paraglegie

Royal Society

Title: Combined effects of anti-Nogo-A antibody treatment and delayed locomotor training in rats with spinal cord injury

Authors: *K. CHEN[1,3], M. COWAN[1], B. C. MARSH[1], C. C. SMITH[1], Y. AL' JOBOORI[1], S. GIGOUT[1], N. GAMPER[1], N. MESSENGER[2], M. E. SCHWAB[4], R. M. ICHIYAMA[1]; [1]Sch. of Biomed. Sci., [2]Ctr. for Physical Educ. and Sports Sci., Univ. of Leeds, Leeds, United Kingdom; [3]Sch. of Biol. Sci. and Med. Engin., Beihang Univ., Beijing, China; [4]Brain Res. Inst., Univ. of Zurich, Zurich, Switzerland

Abstract: An increasing number of studies are being conducted to explore the combined effects of rehabilitation with other strategies for promoting axonal regeneration on the recovery of limb function after spinal cord injury (SCI), believing that tailored combinations of strategies with scientific basis will lead to cumulative improvements in SCI outcomes. When we previously examined the effects of anti-Nogo-A antibody treatment (11C7) and treadmill training in rats with thoracic SCI, their simultaneous application did not show synergistic effects. Since recovery mechanisms of these treatments were not only different but also possibly competitive, the synchronous delivery had likely interfered with each other. We therefore hypothesize that the combinatorial therapy of 11C7 with locomotor training can express positive interactive effects in SCI recovery, if the onset of the training is delayed long enough to minimize the treatments interfering with each other. To test the hypothesis, this study examined the effects of anti-Nogo-

A antibody treatment and two-week delayed onset weight supported locomotor training in a rat model of SCI. All procedures were approved by the UK Home Office. A T-shaped lesion to T9 was made using iridectomy scissors on 25 adult female Sprague-Dawley rats under anesthesia. The animals were divided into 4 treatment groups: 1) non-trained/IgG (n = 7), 2) trained/IgG (n = 6), 3) non-trained/11C7 (n = 6), 4) trained/11C7 (n = 6). Antibody was delivered to the injury site for 2 weeks i.p. with an osmotic pump. Locomotor training started 2 weeks from the end of the antibody treatment. After 8 weeks of locomotor training (5 times/day, 20 min bipedal + 20 min quadrupedal), their behavior and histology were examined. It was found that the trained/11C7 group had better recovery than the other groups in open-field locomotion (mean: 16 vs. 13, BBB score). Training and antibody were shown to improve consistency and dragging in step kinematics, respectively. Antibody reduced the number of errors in inclined climbing. No differences were observed in the nociception assay and lesion volume among the groups. The neuronal activation to induce locomotion in the lumbar spinal cord was reduced with both treatments compared to non-trained/IgG controls (cfos staining). The sprouting supraspinal axons did not show changes far caudal from the lesion site (T13 and L1, BDA staining). Afferent fibers in L2 were increased with antibody but decreased with training (VGLUT/ChAT staining). The results indicate that the combinatorial therapy of 11C7 with delayed training can express positive interactive effects in SCI recovery through refinement of local circuitry.

Disclosures: K. Chen: None. **M. Cowan:** None. **B.C. Marsh:** None. **C.C. Smith:** None. **Y. Al' Joboori:** None. **S. Gigout:** None. **N. Gamper:** None. **N. Messenger:** None. **M.E. Schwab:** None. **R.M. Ichiyama:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.14/V31

Topic: D.10. Spinal Cord Injury and Plasticity

Support: ISRT Grant NRB107

MRC Grant MR\K022911\1

Title: Remodelling of spinal circuits following intraspinal chondroitinase-abc and locomotor training under epidural stimulation following severe contusion injury

Authors: *Y. D. AL'JOBOORI[1], C. C. SMITH[1], K. O. CHEN[1], S. CHAKRABARTY[1], J. W. FAWCETT[2], E. MUIR[3], R. M. ICHIYAMA[1];
[1]Univ. of Leeds, Leeds, United Kingdom; [2]Dept. of Clin. Neuroscience, Cambridge Ctr. for Brain Repair, [3]Dept. of Physiol. Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Combination therapies are likely to be necessary for the treatment of spinal cord injury (SCI). Rehabilitation is routinely used as a clinical treatment and administered early within the sub-acute recovery phase therefore any additional treatment must work synergistically with this. Previous evidence has shown that rehabilitation up-regulates chondroitin sulphate proteoglycan in the lumbar spinal cord likely restricting synaptic plasticity. The current study demonstrates the use of electrical epidural stimulation (ES) of the lumbar spinal cord (L2 to S1), previously shown to improve locomotor function in rat complete transection models in conjunction with bipedal locomotor training and the application of chondroitinase-ABC (delivered in a lentiviral vector; LV-ChABC) in a severe contusion injury of the thoracic spinal cord. Adult Sprague-Dawley rats received a severe spinal contusion injury (T9/10), epidural electrode implantation at segmental levels L2 and S1 and intra-spinal injections of LV-Chase or saline (control). Rats were then randomly assigned to one of four groups: cage control, training only, ES only (40 Hz; L2) or ES+training. Rats in either trained group stepped bipedally/quadrupedally on a body weight supported treadmill (5-16 cm/s) (5 days/week, 20 mins/day) for 8 weeks. Saline+ES+training and ChABC+ES+training groups showed improvement in supported treadmill stepping ability and open field locomotion (BBB). Surprisingly, none of these animals showed any EMG response of hindlimb following cortical stimulation and no increased sensitivity to mechanical pain stimulation. These behavioural results suggest that a combination of step training and epidural stimulation in an incomplete model of SCI successfully improved locomotor function in both treadmill stepping and the open field through changes in local spinal networks. Results from immunohistochemical analysis of synaptic plasticity, axonal sprouting and glial scar morphology will also be discussed.

Disclosures: Y.D. Al'Joboori: None. **C.C. Smith:** None. **K.O. Chen:** None. **S. Chakrabarty:** None. **J.W. Fawcett:** None. **E. Muir:** None. **R.M. Ichiyama:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.15/V32

Topic: D.10. Spinal Cord Injury and Plasticity

Title: The effect of neonatal spinal cord transection on modulation of the monosynaptic reflex during postnatal development

Authors: *C. C. SMITH[1], S. CHAKRABARTY[1], J. F. R. PATON[2], R. M. ICHIYAMA[1]; [1]Univ. of Leeds, Leeds, United Kingdom; [2]Sch. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: Spinal cord injuries in neonates are often accompanied by a greater ability to spontaneously recover function and respond to locomotor treadmill rehabilitation compared to

adults. This is partly due to the remarkable plasticity of the cord caudal to the lesion; however the mechanisms underlying such recovery are poorly understood. The development of locomotion in the rat occurs relatively rapidly with maturity reached after 3 weeks postnatally (PN). During this epoch, the circuitry of the spinal cord establishes and refines its organisation with descending and afferent input competing for innervation in an activity dependant manner. Clarification of how the circuitry of the spinal cord is altered after neonatal injury may offer insight into the mechanism of enhanced recovery. By studying the development of the monosynaptic reflex (MSR), the basic unit of the central pattern generator, we aim to gain insight into how the circuitry of the lumbar spinal cord is altered throughout post natal development in injured and intact animals. We propose that following neonatal spinal cord transection, afferent input becomes the primary modulator of locomotor output and may guide circuit development in a way which promotes recovery of weight bearing locomotion. Mid-thoracic spinal cord transections (tx) were performed on neonates anaesthetised with isoflurane at post natal day 5(PN5). An artificially perfused whole rat preparation was used to illicit MSRs at ages PN10 (5dpi), PN14(9dpi) and PN21 (16dpi). Rats were decerebrated under anaesthesia and then perfused with a modified, oxygenated ringer's solution containing 1.25% ficcol with temperature maintained at 32°C. The tibial nerve was isolated and stimulated using a bipolar hook electrode and ENGs were recorded from the gastrocnemius-soleus (Gs) nerve. Immunohistochemistry was used to assess excitatory and inhibitory inputs to alpha motoneurones (αMN). Compared with intact controls, transected animals exhibited characteristics of increased excitability with reduced paired pulse inhibition, particularly at longer time intervals. This was corroborated by a greater quantity of vesicular glutamate transporter 1 (VG1+) positive terminals on αMNs at PN21. Results suggest that a neonatal transection prevents retraction of Ia afferents from the ventral horn resulting in greater excitatory input to αMNs. These alterations to the normal development of the circuitry of the lumbar spinal cord may contribute to the propensity for recovery afforded to animals injured as neonates.

Disclosures: **C.C. Smith:** None. **S. Chakrabarty:** None. **J.F.R. Paton:** None. **R.M. Ichiyama:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.16/V33

Topic: D.10. Spinal Cord Injury and Plasticity

Support: CREST

KAKENHI on Innovative Areas 15H01434

Title: The pattern of reinnervation of sprouting corticospinal tract fibers after spinal cord injury in macaques

Authors: *H. NAKAGAWA[1], T. NINOMIYA[1], T. YAMASHITA[2], M. TAKADA[1]; [1]Primate Res. Institute, Kyoto Univ., Inuyama, Aichi, Japan; [2]Mol. Neurosci., Osaka Univ., Suita, Osaka, Japan

Abstract: The pattern of intraspinal innervation of corticospinal tract (CST) fibers differs in rodents and primates during development. In addition, manual dexterity strongly relates to the development of the CST in mammals and, therefore, becomes severely impaired after spinal cord injury (SCI). Thus, the sprouting of CST fibers after SCI is critical to recovery of motor functions. In rodents, the CST fibers after SCI sprout beyond the lesion site into the medial gray matter to connect with interneurons. However, the neuroanatomical mechanism underlying the functional recovery through sprouting CST fibers remains unclear in primates. Here we investigated the pattern of reinnervation of CST fibers below the lesion site after SCI in adult macaques. Unilateral lesions were made at the C7/C8 border of the spinal cord. In our SCI model, the dorsolateral funiculus was fully injured to remove laterally-situated CST fibers. The extent of spontaneous recovery of manual dexterity was assessed with a reaching/grasping task. The impaired manual dexterity was recovered gradually over 3 months after SCI. When anterograde tract tracing with biotinylated dextran amine was performed to identify the intraspinal reinnervation of sprouting CST fibers, it was found that their laminar distribution was reorganized. The sprouting CST fibers extended preferentially into lamina IX where the spinal motor neuron pool was located, to innervate the motor neurons directly. Instead, few, if any, CST fibers were distributed in the dorsal laminae. The present results indicate that number of CST fibers sprouting beyond the lesion site after SCI are reorganized for recovery of manual dexterity in macaques.

Disclosures: H. Nakagawa: None. **T. Ninomiya:** None. **T. Yamashita:** None. **M. Takada:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.17/V34

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Grant DA-031197 to M.A. Hook

NIDA Drug Supply Program

Title: Glial activation is necessary for the morphine-induced attenuation of locomotor recovery after SCI

Authors: *M. ACEVES[1,2], A. R. ACEVES[1], S. GONG[1], M. A. HOOK[1,2];
[1]Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Inst. for Neurosci., College Station, TX

Abstract: Opioids are one of few effective analgesics for the treatment of pain following spinal cord injury (SCI). Unfortunately, however, we have shown that morphine administered in the acute phase of SCI, irrespective of the route of administration, compromises recovery of locomotor function, increases mortality and pain reactivity, and suppresses weight gain in a rodent contusion model (Hook et al., 2007, 2009, 2011; Woller et al. 2012, 2014). In order to develop safe and effective strategies for opioid use, we have begun to characterize the cellular changes that accompany morphine treatment after SCI. Our recent studies have shown that the adverse effects of morphine depend on activation of the κ-opioid receptor (KOR), which also appears to be upregulated on astrocytes and microglia after SCI. In the current study we tested whether activation of these glial cells is necessary to produce the adverse effects of morphine. Subjects received a moderate spinal contusion (T12), and an intrathecal cannula was implanted. Baseline locomotor function (BBB) and pain reactivity (tail-flick) were assessed 24 hours following injury. Subjects were then administered minocycline (0, 50, or 100 μg), a glial inhibitor, followed by morphine (0 or 90 μg). Pain reactivity was re-assessed 30 minutes after drug treatment. Recovery was evaluated across a 21-day period, with additional tests of motor and sensory function conducted after day 21. Our results show that pretreatment with minocycline blocked the morphine-induced attenuated recovery, without affecting the acute analgesic effects of morphine. Minocycline may protect against the adverse effects of morphine by decreasing the inflammatory response that is synergistically augmented by opioids and injury. Indeed, the neuroprotective effects of minocycline, an FDA-approved drug, are currently under investigation in multiple clinical trials. Our research suggests that this, and similar drugs, should be further investigated as adjuvants to opioid treatment after SCI.

Disclosures: **M. Aceves:** None. **A.R. Aceves:** None. **S. Gong:** None. **M.A. Hook:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.18/V35

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH NS072651

NIH NS054894

Title: Reawakening trunk motor function with optogenetics in adult spinal transected rats

Authors: *K. A. SCHMIDT, S. F. GISZTER;
Drexel Univ., Philadelphia, PA

Abstract: After a complete T9/T10 spinal cord transection in rats, injury and alteration of motor efferents and sensory afferents force reorganization in the sensorimotor cortex, leaving cortical areas below bregma that previously controlled trunk and hindlimbs silenced to motor function. Rats transected as neonates (NTX) can be trained to weight support (WS) on a treadmill if an elastic field is applied at the pelvis by a Phantom haptic robot. A decrease in the force needed to maintain the elastic field is related to an increase in WS by the rat. These robot/treadmill trained NTX rats show a reorganization of trunk motor areas in cortex, evident from intracortical microstimulation (ICMS) mapping, such that the center of gravity (COG) of the trunk motor area is shifted toward or into areas below bregma (Oza and Giszter, 2015). Since a caudal shift of trunk COG in NTX rats is related to motor recovery after rehabilitation, we asked if we could improve recovery in rats transected as adults (ATX) by inducing plasticity in the trunk motor cortex to 'reawaken' motor areas below bregma using optogenetic subthreshold stimulation. Using viral delivery, we introduced either Channelrhodopsin (CHR2) [AAV5-CamKIIa-hChr2(H134R)-EYFP] or a control fluorophore [AAV5-CamKIIa-EYFP] into pyramidal cells of the sensorimotor cortex below bregma. Three groups of rats were prepared: ATX with ChR2 (ATX-ChR2) current N=3, ATX-ChR2 rats also with AAV5-BDNF injected into the lumbar spinal cord to induce alternation of the hindlimbs (ATX-ChR2/BDNF) current N=3, and a control group (ATX-EYFP) current N=1. All rats were robot/treadmill trained for five weeks for 20min/day during which constant light stimulation was applied from a pair of high powered blue LEDs mounted at craniotomies over cortex below bregma. After five weeks, rats underwent a terminal ICMS map of the motor cortex. A caudal shift in trunk COG was observed in both ChR2 groups (total N=6). The number of cortical sites eliciting trunk motor responses below bregma was increased in both groups compared with ATX maps. Robot force progressively decreased throughout training in the ATX-ChR2/BDNF group as a result of WS stepping improvements. The enhanced plasticity in trunk motor cortex of ChR2 treated rats may facilitate recovery by engaging cortical resources not otherwise available during rehabilitation.

Disclosures: K.A. Schmidt: None. **S.F. Giszter:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.19/V36

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Foundation for Movement Recovery: Oslo, Norway

Title: Human lumbar cord reflex activity to sustained epidural electrical posterior roots stimulation motor behavior during absence and partial presence of brain motor control

Authors: *M. R. DIMITRIJEVIC[1], W. MAYR[2], M. KRENN[2];
[1]Baylor Col. Med., Houston, TX; [2]Univ. of Vienna, Department of Physics and Biomedical Engineering, Austria

Abstract: Human lumbar cord reflex activity to sustained epidural electrical posterior roots stimulation motor behavior during absence and partial presence of brain motor control. Milan R. Dimitrijevic, Matthias Krenn, Winfried Mayr In individuals suffering from complete accidental spinal cord injury (SCI), sustained electrical stimulation of the posterior roots of the lumbar cord disconnected from brain motor control can elicit tonic and rhythmical activity (1). We shall demonstrate the electrophysiological characteristics of mono- and polysynaptic spinal reflex responses elicited by sustained electrical stimulation. Additionally, we include non-reflex configurations of the interneuron network. In incomplete human SCI, the subjects have partial preservation of brain motor control. Studies of the spinal reflex and lumbar cord network activity elicited by epidural stimulation of posterior structures of the lumbar cord show that performing volitional motor tasks has the potential to modify, enhance, and suppress spinal reflex activity and motor behavior. We present a series of interaction characteristics between volitional motor tasks and repetitive spinal reflex response. The recordings show that volitional motor tasks can elicit generalized facilitation or suppression of the excitability of the lumbar network. Future studies may examine localized configurations of the lumbar network. Overall, our findings illustrate that volitional motor tasks and sustained epidural stimulation can improve or replace already existing segmental configuration. [1] Dimitrijevic, M.R., Gerasimenko, Y., and Pinter, M.M. 1998. Evidence for a Spinal Central Pattern Generator in Humans, in Neuronal Mechanisms for Generating Locomotor Activity. Annals of the New York Academy of Sciences, v. 860, pg. 360-376. [2] Jilge B., Minassian K., Rattay F., Pinter MM, Gerstenbrand F., Binder H., and Dimitrijevic M.R. Initiating extension of the lower limbs in subjects with complete spinal cord injury by epidural lumbar cord stimulation. Exp Brain Research (2004) 154: 308-326.

Disclosures: M.R. Dimitrijevic: None. **W. Mayr:** None. **M. Krenn:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.20/V37

Topic: D.10. Spinal Cord Injury and Plasticity

Support: CIHR Grant

Title: Skin-derived precursor schwann cell grafts after complete spinal cord injury in rats

Authors: *Z. MAY[1], R. KUMAR[2], T. FUEHRMANN[3], J. FORERO[1], M. SHOICHET[3], J. BIERNASKIE[2], K. FOUAD[1];
[1]Univ. of Alberta, Edmonton, AB, Canada; [2]Univ. of Calgary, Calgary, AB, Canada; [3]Univ. of Toronto, Toronto, AB, Canada

Abstract: Spinal cord injury (SCI) disrupts the transmission of ascending and descending information from the brain to the spinal cord, leading to motor and sensory dysfunction. Axonal regeneration of spinal tracts is seen as important for the recovery of motor function after SCI. There are many inhibiting factors to regeneration in the CNS, including the formation of a cavity at the injury site. A common approach is to fill the cavity with Schwann cell (SC) grafts to bridge the injury. However, there are many issues associated with culturing Schwann cells from peripheral nerves, including the necessity for an invasive biopsy, the tendency of fibroblasts to overtake cultures, and a lack of SC proliferative capacity. We employ Schwann cells generated from an alternate source: Skin-derived precursors (SKPs), which are multipotent cells found in the dermis. Our experimental goal is to use these SKP-SCs as a scaffold for axonal regeneration after complete transection of the rat thoracic spinal cord. We chose a complete model, because the capacity of SKP-SCs to enhance regeneration in severe models has not been shown, even though patients with tetraplegia make up a meaningful percentage of persons with SCI. Another advantage of a complete model is that it is easier to analyze regeneration, as any axons growing must be regenerated axons. Therefore, in experiment 1, SKP-SCs were transplanted after complete transection of the rat thoracic spinal cord. The rats received one of the following three treatments: lesion only, injection of cell media into the lesion cavity, or injection of GFP+ SKP-SCs into the cavity. After we tried this, we quickly realized the cells did not survive. In our experiments, SKP-SCs were obtained from and grafted into inbred F344 rats, and, thus, immune rejection was not a concern. However, F344 rat sub-strain, *ex vivo* culturing, and viral transduction of the cells may have induced rejection of the graft. Therefore, the aim of experiment 2 was to improve cell survival via immune suppression. Rats received SKP-SC transplants and either daily saline or cyclosporine injections until euthanasia. Cell survival was significantly improved ($p<0.0001$) by administration of cyclosporine (15 mg/kg). Unfortunately the surviving SKP-SCs did not form a bridge across the injury site. Instead, the cells migrated rostral and caudal to the lesion. So, our next goal is to provide a biochemical substrate at the lesion site supportive of cell survival. This project is funded by the Canadian Institutes of Health Research (CIHR).

Disclosures: Z. May: None. **R. Kumar:** None. **T. Fuehrmann:** None. **J. Forero:** None. **M. Shoichet:** None. **J. Biernaskie:** None. **K. Fouad:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.21/V38

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Career Development Award #1 IK2 RX000717-01 from the United States Department of Veterans Affairs, Rehabilitation Research and Development Service

Title: Locomotor stability adaptions in individuals with incomplete spinal cord injury

Authors: *K. E. GORDON[1,2], M. WU[1], G. BROWN[1];
[1]Northwestern Univ. Physical Therapy and Human, Chicago, IL; [2]Res. Service, Edward Hines Jr. VA Hosp., Hines, IL

Abstract: All mechanisms for stabilizing gait are not equal. Individuals with neurologic impairments rely heavily on general stabilization strategies that are present every step to resist potential perturbations. General stabilization strategies (e.g. increasing step width) decrease the necessity of sensing and responding to specific perturbations, but inherently limit speed and decrease energetic efficiency and maneuverability. Thus, training individuals to utilize specific stabilization strategies (e.g. corrective steps) when needed could potentially improve walking ability. However, engaging individuals in practice of specific stabilization strategies is difficult because general stabilization strategies can act as a physiological crutch that overrides the need to make specific corrective actions. Our purpose was to try to reduce reliance on general stabilization strategies during gait in ambulatory individuals with incomplete spinal cord injury (iSCI) by exposing them to a brief period of external lateral stabilization. Two ambulatory subjects with chronic iSCI completed 600 steps of treadmill walking. The first 200 steps were a Baseline measure of unassisted walking. The next 200 steps were done in the presence of a Viscous lateral force field. The field was then removed and subjects walked for another 200 steps to measure After-Effects. Forces were applied to the subjects' hips via cables attached to linear motors. The applied forces varied proportionally to the subjects' lateral center of mass velocities and had the effect of resisting subjects' lateral motion. During the After-Effects period, medio-lateral motion of center of mass, step width variability, and margin of stability variability all increased when compared to Baseline (Figure 1). Step width mean and margin of stability mean both tended to decrease. These results suggest that a temporary period of reduced reliance on general stabilization strategies during gait can be induced in individuals with iSCI by exposing them briefly to external lateral stabilization during walking.

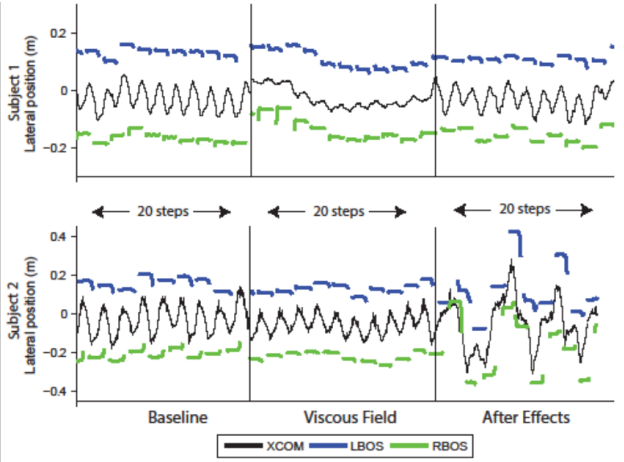


Figure 1: Extrapolated center of mass (XCOM), left base of support (LBOS), and right base of support (RBOS) data for two iSCI subjects walking during Baseline, exposure to a Viscous lateral force field, and After-Effects period immediately after removing the forces. Data shown is for 20 steps at each condition.

Disclosures: **K.E. Gordon:** None. **M. Wu:** None. **G. Brown:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.22/V39

Topic: D.10. Spinal Cord Injury and Plasticity

Support: KAKEN Grant C

Title: The efficacy of functional neuromuscular stimulation using kHz to stimulate gait rhythm in rats following spinal cord injury

Authors: ***T. KANCHIKU**, H. SUZUKI, Y. IMAJO, Y. YOSHIDA, N. NISHIDA, T. TAGUCHI;
Yamaguchi Univ. Grad. Sch. of Med., Ube/Yamaguchi, Japan

Abstract: Background: Rehabilitation facilitates reorganization of residual/regenerated neural pathways, and is key in improving motor function following spinal cord injury. Functional neuromuscular electrical stimulation (NMES) has been reported as being clinically effective. While it can be used from the acute phase post-injury, the optimal stimulation condition to improve motor function remains unclear. Here, we examined the effectiveness of functional electrical stimulation with kHz in gait rhythm stimulation therapy. Methods: Tests were performed using 20 mature female Fischer rats. Incomplete spinal cord injuries (T9 level) were made with an IH impactor at a force of 150 kdyn and NMES was administered for three days from seventh day post-injury. The needle electrodes were inserted percutaneously near the motor

point of each muscle in conscious rats, and each muscle on the left and right leg was stimulated for 15 min, at two frequencies, 75 Hz and 8 kHz, to induce a gait rhythm. Motor function was evaluated using BBB scores and three-dimensional (3D) gait analysis. Rats were divided into four groups (5 rats/group), including the NMES treatment 75 Hz group (iSCI NMES 75Hz), 8 kHz group (iSCI NMES 8 kHz), injury control group (iSCI-NT), and normal group (Normal-CT), and compared. Results: There was no significant difference in BBB scores at any point between the four groups. In 3D gait analysis, compared with the injury control group, the 8 kHz group showed a significant improvement in synergistic movement of both hindlimb. Conclusion: We suggest that kHz stimulation is effective in gait rhythm stimulation using NMES.

Disclosures: **T. Kanchiku:** None. **H. Suzuki:** None. **Y. Imajo:** None. **Y. Yoshida:** None. **N. Nishida:** None. **T. Taguchi:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.23/V40

Topic: D.10. Spinal Cord Injury and Plasticity

Support: The Merit Review Department of the Veterans Affairs

Craig H. Neilsen Foundation

Department of Defense

NYS DOH

Title: Spinal electromagnetic stimulation following spinal cord injury: safety of titanium implants used for spinal stabilization

Authors: *H. A. PETROSYAN[1,3], V. ALESSI[1,3], J. SNIFFEN[2], S. A. SISTO[2], R. DAVIS[4], M. KAUFMAN[3], V. L. ARVANIAN[1,3];
[1]Neurobio. and Behavior, [2]Physical Therapy, Stony Brook Univ., Stony Brook, NY; [3]Northport VA Med. Ctr., Northport, NY; [4]Neurolog. Surgery, Stony Brook Univ. Med. Ctr., Stony Brook, NY

Abstract: In our recent experiments, we demonstrated favorable effects of non-invasive, repetitive spinal electromagnetic stimulation (SEMS) following spinal cord injury in adult rats. SEMS application over intact thoracic spinal cord was able to strengthen transmission and synaptic plasticity in preserved fibers after contusion and hemisection injuries. Chronic administration of SEMS application in combination with exercise induced significant improvements of locomotor function in chronic contused adult rats. The promising properties of this SEMS technique prompted the examination of possible safety concerns for human

applications. Particularly considering that the most spinal cord injured patients have implanted metal devices used for spinal stabilization. In this study, we examined the effects of SEMS on potential heating and displacement of titanium rods used for spinal stabilization. Using adult rats, we have also examined possible effects of implanted titanium rods on motor-evoked potentials (MEPs) recorded from hindlimb muscles, and other physiological parameters such as heart rate and respiratory rate. Magstim-Rapid^2 stimulator with figure of eight D-70^2 coil was used in this study. Maximum intensity (100%) was used to examine possible temperature changes and displacement of titanium rods (used in Stony Brook Hospital for spinal stabilization), and similar rods made of other metals, such as stainless steel, aluminum and copper. Potential heating of rods exposed to electromagnetic stimulation was examined by recording the rod temperature, coil temperature and ambient temperature surrounding rods and coil using high sampling rate thermocouple probes and infrared thermometers. Potential displacement of rods was examined using a force transducer to measure force exerted on the rods during electromagnetic stimulation. To examine possible effects of SEMS on MEPs and physiological parameters, two titanium rods were implanted bilaterally at the thoracic level in adult rats and MEPs, heart rate and respiratory rate were recorded both with and without titanium rods during SEMS application. Results demonstrate that SEMS does not induce any discernable movement and temperature changes in titanium rods. The presence of the rods did not affect MEPs and the threshold intensity to evoke MEPs. Heart rate and respiratory rate were also unaffected. In addition, a post-mortem examination of the spinal cord and surrounding tissues revealed absence of SEMS induced physical damage. These results suggest that SEMS application, even with the presence of spinal titanium implants, does not induce adverse effects and thus may be a safe approach for patients with SCI

Disclosures: **H.A. Petrosyan:** None. **V. Alessi:** None. **J. Sniffen:** None. **S.A. Sisto:** None. **R. Davis:** None. **M. Kaufman:** None. **V.L. Arvanian:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.24/V41

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Foundation for Physical Therapy PODS I Scholarship (TDF)

Foundation for Physical Therapy Florence P. Kendall Scholarship (TDF)

NIH 1 R01 NS074882-01A1 (DMB)

Title: Defining a novel model of sensorimotor impairment after spinal cord injury

Authors: *T. D. FAW[1,2,3,4], J. K. LERCH[1,3,5], S. D. KERR[1,3,4], R. J. DEIBERT[1,3,4], L. C. FISHER[1,3,4], D. M. BASSO[1,3,4];
[2]Neurosci. Grad. Program, [3]Ctr. for Brain and Spinal Cord Repair, [4]Sch. of Hlth. and Rehabil. Sci., [5]Dept. of Neurosci., [1]The Ohio State Univ., Columbus, OH

Abstract: Neuropathic pain is a devastating and often intractable consequence of SCI in up to two-thirds of patients with many reporting a negative impact on quality of life (QOL). Here we report divergent sensorimotor recovery after SCI in a transgenic mouse line that, for the first time, allows differential study of sensory pathways involved in neuropathic pain. This model will provide valuable molecular targets for treatment of sensory dysfunction after CNS injury. Severe SCI contusion (SCI) or transection (TX) was performed at T9 in Thy1-EGFP/M+ (TG+; SCI n=12, TX n=3), Thy1-EGFP/M- (TG-; SCI n=4) littermates, and C57BL/6 (WT; SCI n=10) mice. Thermal and mechanical sensation was examined prior to and 42 days post injury (dpi) using Plantar Heat and Von Frey Hair Test, respectively. Motor recovery was evaluated in the open field using the Basso Mouse Scale pre-injury, 1 dpi, and weekly to 42 dpi. Precise hindlimb motor control during locomotion was tested on a grid walkway. Percent tissue sparing at the epicenter indicated lesion severity. TG+ and TG- SCI groups had significantly greater locomotor performance in the open field compared to historic WT SCI controls by 7 dpi (WT SCI = 2.4±0.7, TG+ SCI = 4.8±0.5, TG- SCI = 5.0±0.0; $p<.01$) and remained higher at 42 dpi (WT SCI = 5.6±0.4, TG+ SCI = 6.5±0.3, TG- SCI = 7.5±0.0; $p<.01$). Precise paw placement was severely impaired in TG+ and TG- animals (% success; TG+ SCI Pre = 97.2%±1.0, Post= 6.8±2.5; TG- SCI Pre = 98.8±1.3, Post= 5.8±1.8). Thermal hyperalgesia occurred following SCI and TX in TG+ and TG- groups (TG+ SCI Pre = 9.2sec±1.0, Post = 5.5±0.7; TG- SCI Pre = 7.6±2.0, Post = 2.3±0.5; TG+ TX Pre = 7.9±0.8, Post = 4.0±0.3; $p<.001$). Interestingly, mechanical hypoalgesia rather than allodynia emerged after SCI in both TG+ and TG- groups (TG+ SCI Pre = 0.37g±0.06, Post = 0.89±0.12; TG- SCI Pre = 0.75±0.10, Post = 2.18±0.61; TG+ TX; Pre = 0.67±0.17, Post = 4.33±0.33; $p<.001$). Tissue sparing was also greater in TG+ SCI animals (WT SCI = 12.9%±2.3; TG+ SCI = 38.8±2.5; $p<.001$). Using clinically-relevant SCI, we describe an innovative genetic model which demonstrates hypersensitivity to pain yet hyposensitivity to touch. This divergent profile allows a unique opportunity to examine mechanisms underlying sensory recovery essential to improving QOL for people with SCI. Current experiments aim to determine the location and impact of the Thy1-EGFP transgene insertion and associated mutations.

Disclosures: T.D. Faw: None. **J.K. Lerch:** None. **S.D. Kerr:** None. **R.J. Deibert:** None. **L.C. Fisher:** None. **D.M. Basso:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.25/V42

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Spinal Cord Injury Treatment Centre (Northern Alberta) Society

Alberta Paraplegic Foundation

Title: Improving walking and inducing neuroplasticity after chronic SCI by training in the ReWalk exoskeleton

Authors: *A. KHAN[1], D. LIVINGSTONE[2], J. MISIASZEK[3], R. STEIN[4], M. GORASSINI[5], P. MANNS[2], J. YANG[2];
[1]Neurosci. and Mental Hlth. Inst., [2]Physical Therapy, [3]Occup. Therapy, [4]Physiol., [5]Biomed. Engin., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Recent developments in powered exoskeletons such as the ReWalk have shown promise to restore walking ability in people with severe spinal cord injury (SCI). Objective: Determine the effects of training in the ReWalk exoskeleton on walking function and neuroplasticity. Participants were individuals with complete or incomplete SCI, ≥1 yr post-injury, with arm strength to control forearm crutches, and who use the wheelchair as their primary mode of mobility. Training consisted of ~1 hr/day, 5 days/wk for 12 wks, progressing from sit-to-stand, stand-to-sit, balancing in standing, walking on smooth ground, turning while walking, to walking on uneven ground, ramps, curbs and stairs. Measurements were taken 2-3 times at baseline, then 6 and 12 weeks into training. Walking function included the maximum walking distance without a rest, the 10-m walk test, and the 6-min walk test (6MWT). Effort of walking was estimated with the physiological cost index (PCI) during the 6MWT. Postural stability was measured on a force platform, including the limits of stability (area enclosed by maximum leans in 8 directions) and postural sway with eyes open and closed. Plasticity in the motor pathways was determined by single-pulse, transcranial magnetic stimulation to induce motor evoked potentials in the paravertebral muscles. Spasticity was estimated with the Spinal Cord Assessment Tool for Spasticity and the cutaneomuscular reflex induced by stimulation of the posterior tibial nerve. Plasticity in sensory pathways was determined with the electrical sensory perceptual threshold. Pain was estimated weekly using the McGill Pain Questionnaire. A field test was conducted towards the end of training to determine the feasibility of the ReWalk for use in the home and community. Results: All participants were able to achieve over ground walking of ~1 km without a rest after ~50 sessions of training. Walking speeds ranged from 0.3 to 0.5 m/s in the ReWalk at the end of training. PCI was <3 heart beats/m (HB/m) during the 6MWT (uninjured: 0.6 HB/m; with hip-knee-ankle-foot orthosis after complete SCI: ~14 HB/m). All participants showed dramatic improvements in postural stability. Some individuals showed plasticity in motor and sensory pathways, and reduction in pain and spasticity. Most participants could walk easily indoors and outdoors with the device, but stairs, curbs and other tasks were difficult. Conclusion: These preliminary results suggest ReWalk training enabled over ground ambulation for individuals with severe SCI, and generated useful neuroplasticity. We believe it is a good device to incorporate into the retraining of walking and balance after severe SCI.

Disclosures: **A. Khan:** None. **D. Livingstone:** None. **J. Misiaszek:** None. **R. Stein:** None. **M. Gorassini:** None. **P. Manns:** None. **J. Yang:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.26/V43

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation

NS 054894

Drexel College of Medicine Dean's Fellowship for Excellence in Collaborative and Themed ResearchDrexe

Title: Investigating combination therapies of robot-driven epidural stimulation, robot rehabilitation, and viral delivery of Brain-derived neurotrophic factor (BDNF) in treating adult spinal cord injury (SCI)

Authors: *J. LEE, S. F. GISZTER;
Neurobio. & Anat., Drexel Univ., Philadelphia, PA

Abstract: A complete transection at T10 in an adult rat (ATX) is a useful model to investigate locomotor rehabilitation in SCI. Based on previous work in our lab, we employ a unique trunk-based robotic intervention focused at a rat's pelvis to significantly improve locomotor function with treadmill training in various treatment paradigms: animals transected as neonates (NTX), ATX animals treated with AAV5 delivery of BDNF, and with epidural stimulation. Our prior work showed that nearly 30% of ATX rats treated with AAV5-BDNF develop hyperreflexia after initial training improvements, leaving them functionally unable to fully alternate and extend their hindlimbs. We hypothesized that we can mitigate this "collapse" of function with the use of robot-driven epidural stimulation. However, it may also exacerbate the collapse and/or its frequency. To test the effects of this combination therapy on outcome, we designed a treatment regimen combining epidural stimulation, robot rehabilitation, and AAV5-BDNF. We prepared two groups of rats (current n=3/group) with stimulating electrodes placed on the surface of the spinal cord at L2 and S2, and with microinjections in the spinal cord caudal to the transection site: one group received AAV5-BDNF, and the other received a sham virus. After recovery, animals were treadmill trained with robotic pelvic rehabilitation therapy and epidural stimulation for six weeks. To examine the effects of combination therapy on locomotor recovery, we compared stepping measures and stimulation parameters between the two groups. Specifically, we examined (1) stimulation intensity needed to induce locomotor activity, and (2) the threshold to induce locomotor activity as a function of time over weeks of recovery. The group treated

with AAV5-BDNF showed a marked decrease in stimulation intensity to induce locomotion, and these effects lasted throughout recovery. Thus far epidural stimulation is not inducing increased collapse of rehabilitation - no rats have shown this in the combined groups. However, the numbers of rats are not yet at criterion levels to support a conclusion. With the emergence of neuroprosthetics and other combined therapies, we believe this work provides a model for testing interactions between robotic, bionic, and viral biological therapies in the treatment of SCI.

Disclosures: J. Lee: None. **S.F. Giszter:** None.

Poster

338. Spinal Cord Injury II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 338.27/V44

Topic: B.02. Ligand-Gated Ion Channels

Title: Responses of CSF-contacting cells in the spinal cord to activation of purinergic receptors

Authors: *C. MACLEAN[1], J. DEUCHARS[1], L. PEERS[1], S. LARRINGTON[1], N. COHEN[2], S. A. DEUCHARS[1];
[1]Sch. of Biomed. Sci., [2]Sch. of Computing, Univ. of Leeds, Leeds, United Kingdom

Abstract: The central canal region of the spinal cord may be considered to be one of the adult neurogenic niches and there are well documented increases in proliferation of the ependymal cells in this area after injury (Lacroix et al, 2014). Other cell types are also present in this area including the CSF-contacting cells (CSFcCs) that send a process into the central canal. In mammals, the role of these cells has yet to be fully elucidated but they may be important in signalling the response to injury. This study examines responses of CSFcCs to activation of purinergic receptors as these have previously been implicated in damage recognition systems in other cell types (Lahne and Gale, 2010) and P2X receptors containing the $P2X_2$ subunit are expressed by the CSFcCs present in this area (Stoeckel et al, 2003). Anaesthetised 11 day old Wistar rats were perfused with sucrose aCSF, followed by sectioning of thoracic/lumbar spinal cord regions at 300 μm with a vibrating microtome. Whole-cell patch clamp electrophysiology was used to obtain single cell recordings from CSFcCs in these spinal cord slices. ATP was applied using a puff electrode connected to a PicoPump. Post-recording and post-fixation visualisation was enabled by inclusion of a fluorescent and a DAB-reactive dye, respectively, to confirm cell type classification. Local application of ATP (300 μM) elicited fast depolarisations in both spiking and non-spiking subtypes of CSFcC (types 1, 2 and 3 (Corns et al, 2013)). Bath application of the broad-spectrum purinergic antagonist Suramin (50 μM) reduced the magnitude of these responses from 21.50 ± 5.16 mV to 9.73 ± 3.03 mV and delayed the onset of the depolarisations. The $P2X_{2/3,\ 3}$-specific antagonist A317491 (1 μM) had no effect in one group, produced partial reduction in magnitude of depolarisation in a second group and complete

elimination of depolarisation in the remaining CSFcCs. Immunohistochemical analysis of the P2X subtypes in this area revealed a sub-population of CSFcCs, predominantly in the ventral area of the region around the central canal that expressed $P2X_3$ in addition to $P2X_2$-containing receptors indicated by previous research. The presence of fast acute responses to ATP and spatial variation in receptor subtypes suggests a role for purinergic signalling in the functioning of the CSFcCs in this area that may include responding to damage.

Disclosures: **C. Maclean:** None. **J. Deuchars:** None. **L. Peers:** None. **S. Larrington:** None. **N. Cohen:** None. **S.A. Deuchars:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.01/V45

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R24-HD50821-07

Title: Hemispheric stroke induces major structural changes in paretic muscles

Authors: ***M. K. CHARDON**, Y. DHAHER, N. SURESH, W. Z. RYMER;
SMPP, Rehabil. Inst. of Chicago, Chicago, IL

Abstract: In addition to altering motor control, stroke-induced spastic paresis is believed to alter the architecture of impaired skeletal muscles. Describing such changes is important in our understanding of the mechanisms of stroke-induced muscle impairment. At the musculotendon unit level, descriptions of passive mechanics have thus far only been addressed obliquely, to the best of our knowledge. Spastic Achilles tendon was shown to be longer and softer while we reported a stiffer spastic musculotendon unit using tendon indentation (Zhao 2009; Chardon 2014, 2010). A full description of the passive mechanical properties of the musculotendon unit is thus needed. We set out to measure these mechanical properties in passive musculotendon units of chronic stroke survivors and controls. Using in combination, an ultrasound machine and a position controlled tendon indenter, we recorded ultrasound movies of the biceps brachii as its distal tendon was being mechanically indented. The indentation force at the tip of the indenter was also recorded. The mechanical properties were initially extracted by tracking features found along the proximal to distal axis of the biceps brachii throughout all frames of the ultrasound movies. We then related the motion of the features to the indentation force. We distilled this data to two parameters: the Transition Point which is the indentation location at which the motion switches from a non-linear to linear behavior and the Stiffness. We report that the affected musculotendon unit of our stroke cohort has a statistically lower transition point and a greater stiffness than the contralateral and control. On aggregate the affected side will transition 61%

and 50% sooner and is 40% and 57% stiffer than the contralateral and control. We also report that in contrast to the contralateral and control, the transition point and stiffness of the affected musculotendon unit are statistically invariant throughout the ultrasound imaging window. The affected musculotendon unit thus appears to be homogenous. Using this novel method, we report in-vivo a clear demarcation of the affected musculotendon unit passive mechanics from the others. Furthermore, we show that these differences are also structural in that the affected side has a homogenous mechanical behavior. In other words for the inhomogeneous passive mechanical properties of healthy skeletal muscle to disappear, stroke-induced spastic paresis must fundamentally rearrange the internal architecture of the musculotendon unit. As to which mechanism is responsible for the rearrangement, many point to fibrosis however more evidence is needed.

Disclosures: M.K. Chardon: None. **Y. Dhaher:** None. **N. Suresh:** None. **W.Z. Rymer:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.02/V46

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The impact of altering cholinergic activity on nmjs in normal and stress conditions

Authors: *S. SUGITA[1], L. FLEMING[2], G. VALDEZ[3];
[1]Virginia Tech., Roanoke, VA; [2]Biol. sciences, Virginia Tech., Blacksburg, VA; [3]VTCRI, Virginia Tech., Roanoke, VA

Abstract: The motor system undergoes significant changes with age and the progression of diseases, in part due to dysregulation of synaptic function. In this study, we sought to determine the impact of increasing and decreasing cholinergic neurotransmission on aging and ALS-afflicted neuromuscular junctions (NMJ). We examined transgenic animals for the vesicular acetylcholine transporter (VAChT), a protein required to package acetylcholine into synaptic vesicles. These mice have higher (VAChT-Hyper) or lower (VAChT-KD) acetylcholine levels and release at synaptic sites. We first asked if altering cholinergic activity impacts the normal development of NMJs. Using light microscopy, we found no obvious differences in the size, architecture and rate of synaptic elimination between NMJs in 9 days-old VAChT-Hyper and control mice. In young adult mice, however, we discovered age- and disease-related structural and molecular alterations. Many NMJs were found denervated and fragmented in VAChT-Hyper transgenic compared to control mice. Not surprisingly, $SOD1^{G93A}$ mice overexpressing VAChT die prematurely. In contrast, NMJs appear structurally normal in VAChT-KD mice. Additionally, $SOD1^{G93A}$ with reduced levels of VAChT live longer compared to $SOD1^{G93A}$ mice.

These results indicate that fine tuning cholinergic activity in muscles may be a therapeutic approach to slow down the progression of age and ALS-related motor deficits.

Disclosures: **S. Sugita:** None. **L. Fleming:** None. **G. Valdez:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.03/V47

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH

Title: Muscle fiber length-dependent, intramuscular variation in damage-induced remodeling of neuromuscular junctions in a mouse model of muscular dystrophy

Authors: *Y. LEE[1], R. MASSOPUST[2], W. J. THOMPSON[1,2];
[1]Dept. of Biol., [2]Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: Duchene muscular dystrophy (DMD), a recessive X-linked degenerative muscle disease, results from missense mutations within the dytrophin gene. In addition to the well-documented segmental necrosis of skeletal muscle fibers, there is a loss of force generation by muscles and alterations in the morphology of the neuromuscular junction (NMJ) - the site of synaptic communication from a motor neuron to a skeletal muscle fiber. These alterations include “fragmentation” of postsynaptic neurotransmitter receptor (acetylcholine receptor; AChR) aggregates that may further compromise proper functioning of the neuromuscular system, and be a compounding factor in DMD pathology. An overwhelming body of evidence supports the hypothesis that lack of functional dystrophin protein leads to muscle fiber fragility - and subsequent cycles of damage and regeneration. The mechanism(s) responsible for alterations of the NMJs, however, remain controversial. It has been argued that dystrophin contributes to synaptic aggregation of AChR, although evidence also exists to support the idea that junctional alterations arise primarily as a result of damage and regeneration of synaptic segments of muscle fibers. Our examination of an expiratory muscle, triangularis sterni (TS) in a mouse model (mdx) revealed that, at ~5 weeks of age, only a minority of NMJs have the fragmented morphology associated with dystrophy; the remainder of the junctions appear normal and indistinguishable from those found in control muscles. This is in stark contrast to the TS of ~9 weeks-old mdx, where most junctions appear fragmented. Interestingly, in both age groups, there are clear differences between NMJs at anterior and posterior portions of the muscle in the degree to which the NMJ morphology is altered. Such a gradient in morphological alterations may be functionally correlated to the lengths of muscle fibers in TS - the shortest and longest fibers at anterior and posterior ends of the muscle, respectively. In addition, there appear to be, at a subset of

fragmented NMJs, an increased number of terminal Schwann Cells (tSCs) with altered morphology. Our preliminary findings, thus, suggest that 1) NMJs are formed normally in absence of functional dystrophin, 2) junctional alteration progresses against the known anterior-posterior gradient in neuromuscular maturation and 3) tSCs, which participate in sculpting of neonatal endplates, may become activated in response to muscle fiber injury.

Disclosures: Y. Lee: None. **R. Massopust:** None. **W.J. Thompson:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.04/V48

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant

Title: Pre- and postsynaptic changes at the neuromuscular junction in two models of duchenne muscular dystrophy

Authors: *S. HADDIX[1], Y. LEE[2], J. N. KORNEGAY[3,1], W. THOMPSON[1,2];
[1]Inst. for Neurosci., [2]Dept. of Biol., [3]Col. of Vet. Med. and Biomed. Sci., Texas A&M Univ., College Station, TX

Abstract: Duchenne muscular dystrophy is an X-linked disease that causes a loss of expression of dystrophin. This protein is a key component of the dystroglycan complex providing a link between the contractile apparatus of myofibers and the extracellular matrix, stabilizing the sarcolemma. As such, dystrophin loss results in increased damage to contracting muscle. It is also well documented that dystrophy leads to postsynaptic changes at the NMJ, whose ultimate cause remains elusive. To assess the underlying mechanisms of synaptic change, we examined NMJs in dogs (GRMD) and *mdx* mice that have dystrophin gene mutations leading to dystrophy. By fluorescently staining GRMD muscles for acetylcholine receptors (AChR), neurofilament and synaptic terminal markers, Schwann cell (SC) proteins, and acetylcholine esterase (AChE) we recorded morphological changes of dystrophic NMJs. Abnormalities to AChR endplate structure that mirror the *mdx* mouse were observed. Many dystrophic endplates are dispersed into fragments and are larger when compared to pretzel-shaped controls. The similarity to junctional alterations in muscles treated with myotoxic drugs suggests that a process of myofiber degeneration and regeneration, a hallmark of dystrophic muscle pathology, is producing these synaptic changes. Furthermore, the alterations of the postsynaptic apparatus correlate with changes to the presynaptic structure. In particular, the axon terminal is more branched as compared to controls. Additionally dystrophic NMJs have more terminal SCs apposing the endplate. It is possible that these phenomena may have roles in restructuring the postsynaptic

surface following degeneration and regeneration of the muscle fiber. Interestingly the presynaptic structure appears to remain in place during muscle fiber necrosis. This is evidenced by areas that stain highly for nerve and AChE, but lack corresponding AChR stain. As the axon itself is known to have roles in receptor clustering via agrin signaling, a simple hypothesis for fragmentation and enlargement of the endplate is that the axon terminal at some point makes additional contacts to the myofiber, forming new aggregates. Additionally, terminal SCs have been shown to be capable of altering synaptic morphology, which may tie into the changes seen at dystrophic endplates. Finally, adjacent muscle fibers in GRMD are sometimes innervated by the same axon suggesting motor unit size increase. As many of these changes are also observed in the *mdx* mouse, further research will use this model to investigate what the exact cause of endplate fragmentation and enlargement is, and whether it contributes to the dystrophic disease state.

Disclosures: **S. Haddix:** None. **Y. Lee:** None. **J.N. Kornegay:** None. **W. Thompson:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.05/W1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS064224

MDA Grant (MDA4209)

TTUHSC Seed Grant 14029IB

Title: Spinal muscular atrophy amelioration by genetic inhibition of the JNK pathway

Authors: *L. D. GANGWANI, N. K. GENABAI, Z. ZHANG, X. JIANG;
Biomed. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., El Paso, TX

Abstract: Mutation of the Survival Motor Neurons 1 (SMN1) gene causes spinal muscular atrophy (SMA), an autosomal recessive early childhood neurodegenerative disorder. Degeneration of spinal motor neurons caused by SMN deficiency results in progressive muscle atrophy and death in SMA. The molecular mechanism underlying neurodegeneration in SMA is unknown. No treatment is available to prevent neurodegeneration and reduce the burden of illness in SMA. We report that the c-Jun NH2-terminal kinase (JNK) signaling pathway mediates neurodegeneration in SMA. The CNS specific isoform JNK3 is required for neuron degeneration caused by SMN deficiency. JNK3-deficiency reduces degeneration of cultured neurons caused by low levels of SMN. Genetic inhibition of JNK pathway *in vivo* by Jnk3 knockout results in amelioration of SMA phenotype. JNK3-deficiency prevents the loss of spinal cord motor

neurons, reduces muscle degeneration, improves growth, motor function and increases lifespan of mice with SMA that shows a systemic rescue of phenotype by SMN-independent mechanism. JNK3 represents a potential (non-SMN) therapeutic target for the treatment of SMA.

Disclosures: L.D. Gangwani: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Filed a patent application on use of JNK inhibitors for treatment of SMA. **N.K. Genabai:** None. **Z. Zhang:** None. **X. Jiang:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.06/W2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Kennedy's Disease Association

Title: Changes in polyglutamine repeat length alter disease progression in a mouse model of Kennedy's Disease

Authors: J. ZENCHAK[1], M. ALTEMUS[1], *J. A. JOHANSEN[2];
[1]Neurosci. Program, [2]Col. of Med., Central Michigan Univ., Mount Pleasant, MI

Abstract: Kennedy's disease (KD, also known as spinal bulbar muscular atrophy) is a neurodegenerative disorder that exhibits myopathic as well as neuropathic characteristics, which include progressive muscle weakness, atrophy, and a loss of motor neurons in the spinal cord and brain stem. The molecular basis of this disease is an expansion in the number of CAG repeats located in the androgen receptor (AR) gene that will result in a misfolded and dysfunctional protein. One popular mouse model to study KD is the AR113Q model. This model contains 113 CAG repeats in the androgen receptor gene resulting in a loss of motor function and early death. Unfortunately, the number of repeats contracts in each generation in this mouse model. The purpose of this study was to determine whether AR113Q mice with a contracted CAG repeat length ranging from 90-92 will still exhibit a loss of motor function. In this study, 24 male mice (n=12 wt and n=12 tg) were subjected to a series of behavioral tests (open field, grip strength and stride length) from three to seven months of age. No significant differences were found between the wt and tg mice at these ages. However, tg mice with 90-92 CAG repeats exhibited early death, with mean age of survival at 17.5 weeks. This suggests that the contracted AR may still be toxic, but that longer repeat lengths may be required to observe motor deficits.

Disclosures: J. Zenchak: None. **M. Altemus:** None. **J.A. Johansen:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.07/W3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR RN120654 - 244847

NSERC 217494-2013

Title: Decreased activity and increased ubiquitin-mediated degradation of human choline acetyltransferase by mutation of an N-terminal proline-rich motif

Authors: *T. M. MOREY[1], S. ALBERS[1], B. SHILTON[2], R. J. RYLETT[1];
[1]Physiol. and Pharmacol., [2]Biochem., Western Univ., London, ON, Canada

Abstract: Choline acetyltransferase (ChAT) is essential for the function of cholinergic neurons as it mediates synthesis of the neurotransmitter acetylcholine. Mutations within ChAT have been linked to congenital myasthenic syndrome (CMS), a rare neuromuscular disorder characterized by cholinergic dysfunction of the neuromuscular junction. One CMS-related ChAT mutation, Val18Met, reduces both enzyme activity and protein steady-state levels. Interestingly, though residue Val18 is not located within the ChAT active-site, it is located within a highly-conserved, surface-exposed proline-rich motif at residues 14-PKLPVPP-20 that shares homology with SH3-domain binding motifs. It is currently unknown if this motif regulates ChAT function. In the present study, we demonstrated that N-terminal truncation that includes this proline-rich motif, as well as mutation of both residues proline-17 and -19 to alanine (P17A/P19A) dramatically reduces ChAT steady-state protein levels (80%) and produces a catalytically-inactive enzyme compared to wild-type (WT) ChAT when expressed in cholinergic SN56 neural cells. The *in vitro* specific activity of bacterially-expressed recombinant P17A/P19A-ChAT is also reduced (76%), though this is not due to gross changes in protein secondary structure or thermal stability as measured by circular dichroism. Using a novel fluorescent-biorthogonal pulse-chase protocol in SN56 cells, we determined that the half-life of P17A/P19A-ChAT protein (2.2 hours) is substantially reduced when compared to WT-ChAT (19.7 hours). By using heterologously-expressed HA-tagged ubiquitin, we found that ChAT is ubiquitinated and that polyubiquitination of P17A/P19A-ChAT is increased when compared to WT-ChAT. Further investigation reveals that both WT and P17A/P19A-ChAT can undergo proteasomal degradation through both lysine-48 (K48)-linked and K48-independent polyubiquitination. Lastly, treating SN56 cells with the proteasome inhibitor MG132 increases P17A/P19A-ChAT half-life (16.8 hours) and steady-state protein levels when compared to DMSO-control, though failed to restore its catalytic activity. These results identify a novel mechanism for the regulation of cellular ChAT protein levels through the ubiquitin-proteasome system that is influenced by the conserved N-terminal proline-rich motif of ChAT. T.M.M is a recipient of an Ontario Graduate Scholarship.

Disclosures: **T.M. Morey:** None. **S. Albers:** None. **B. Shilton:** None. **R.J. Rylett:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.08/W4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MDA Grant (MDA186316)

Seed Fund for Basic Science Research from National University Health System, Singapore (2013/183/T1-BSRG/09)

Title: A novel cell-based serological assay for myasthenia gravis using Xenopus tissue cultures

Authors: ***C. LEE**, H. L. YEO, J. Y. LIM;
Dept. of Physiol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Myasthenia gravis (MG), the most common autoimmune disease of neuromuscular junction (NMJ), is heterogeneous in terms of pathophysiology, which is determined by the pathogenic antigen of autoantibodies targeting to synaptic proteins at the NMJs. Currently, patients suspected with MG are routinely screened for the presence of autoantibodies against acetylcholine receptor (AChR) or muscle-specific kinase (MuSK) using a cell-based assay (CBA) that involves the expression of target synaptic membrane protein in heterologous cell lines. However, some autoantibodies may only show reactivity for binding to densely clustered AChR in the physiological conformation, while AChR clustering is known to involve signaling events orchestrated by over a dozen of postsynaptic proteins. To improve the existing serological diagnosis of MG, this study explored the possibility of using the well-established Xenopus primary culture system as a novel CBA for MG. Here, by examining the pathogenic effects of four MG human plasma samples, we found that the samples from both seropositive and seronegative MG patients effectively induced the disassembly of aneural AChR clusters in cultured Xenopus muscle cells, as well as the nerve-induced AChR clusters in the nerve-muscle co-cultures. Importantly, the disassembly of AChR clusters was spatio-temporally correlated to the disappearance of actin depolymerizing factor (ADF)/cofilin, an actin regulator involved in AChR trafficking and clustering. Taken together, this study develops a reliable CBA using Xenopus primary cultures for screening the pathogenicity of human MG plasma samples, and providing a platform for investigating the pathogenic mechanisms underlying the endocytic trafficking and degradation of AChRs at NMJs in MG patients.

Disclosures: **C. Lee:** None. **H.L. Yeo:** None. **J.Y. Lim:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.09/W5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Early detection and characterization of neurological function in SOD1 G93A mice

Authors: I. MORGANSTERN, B. FERETIC, K. HOMA, S. A. MALEKIANI, M. NILGES, N. E. PATERSON, N. ROBERTS, E. SABATH, G. SARDARYAN, L. THIEDE, *T. HANANIA; PsychoGenics Inc., Tarrytown, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Transgenic mice over-expressing human SOD1-G93A are commonly employed as an animal model of familial ALS. The present study was aimed at identifying behavioral tests that are most sensitive to the emergence of behavioral/neurological deficits in male and female SOD1 G93A mice (SOD-1) compared to wild-type (WT) controls. Specifically, the test battery consisted of commonly used metrics such as fore- and hind-limb grip strength, rotarod, open field and more complex proprietary algorithm-based behavioral platforms such as NeuroCube® and SmartCube® Systems. The results of the present study indicated that both fore- and hind-limb grip strength as well as rotarod performance declined with age and disease progression. The open field test revealed time-dependent changes in locomotor and rearing performances in SOD-1 mice of both genders, with relatively stable performance in WT controls. In addition to these routine tests of neurological and motor function, sophisticated algorithm-based systems were employed and determined a strong phenotype effect in the SOD-1 mice at a much earlier age. Specifically, by using the NeuroCube® system to measure gait deficits, we found that SOD-1 mice showed a reduction in step and stride length and an increase in stride, stand and swing duration compared to WT mice, which was evident as early as 8 weeks and progressed with age. Similarly, by using our SmartCube® technology that measures whole animal behavior, we identified behavioral changes as early as 6-7 weeks of age in the SOD-1 mice. Interestingly, at young ages the mutant mice showed a unique hyperactive phenotype consisting of increased mobility, exploratory behavior, grooming and sniffing compared to WT mice which declined with disease progression. In summary, the platform of behavioral tests employed here demonstrate that while routine behavioral paradigms are able to detect neurological and motor function deficits in SOD-1 versus WT mice as early as 13-15 weeks of age, more advanced computer vision systems are able to identify distinctive behavioral patterns and discriminate the phenotype as early as 6-8 weeks of age. This earlier period of disease identification presents a valuable model in which to explore and improve future assessment of potential therapeutic approaches for ALS.

Disclosures: **I. Morganstern:** None. **B. Feretic:** None. **K. Homa:** None. **S.A. Malekiani:** None. **M. Nilges:** None. **N.E. Paterson:** None. **N. Roberts:** None. **E. Sabath:** None. **G. Sardaryan:** None. **L. Thiede:** None. **T. Hanania:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.10/W6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01NS066927

NIH R21NS081364

NSFC 81129019

Title: Reactivation of lysosomal calcium efflux rescues abnormal lysosomal storage in fig4 deficiency

Authors: ***J. LI**[1,2,3], J. ZOU[1], B. HU[1], S. ARPAG[1], Q. YANG[1], A. HAMILTON[1], Y. ZENG[2], C. VANOYE[3];
[1]Dept. of Neurol., Vanderbilt Univ., Nashville, TN; [2]Sun Yat-sen Univ., Guangzhou, China; [3]Northwestern Univ., Chicago, IL

Abstract: Loss-of-function of FIG4 leads to Charcot-Marie-Tooth disease type-4J, Yunis-Varon syndrome, or an epilepsy syndrome. FIG4 is a phosphatase with its catalytic specificity towards 5'-phosphate of phosphatidylinositol-3,5-diphosphate (PI3,5P2). However, the loss of FIG4 decreases PI3,5P2 levels likely due to FIG4's dominant effect in scaffolding a PI3,5P2 synthetic protein complex. At the cellular level, all these diseases share similar pathology with abnormal lysosomal storage and neuronal degeneration. Mice with no FIG4 expression (Fig4-/-) recapitulate the pathology in humans with FIG4 deficiency. Using a flow cytometry technique that rapidly quantifies lysosome sizes, we detected an impaired lysosomal fission, but normal fusion, in Fig4-/- cells. The fission defect was associated with a robust increase of intralysosomal Ca2+ in Fig4-/- cells, including FIG4-deficient neurons. This finding was consistent with a suppressed Ca2+-efflux of lysosomes, since the endogenous ligand of lysosomal Ca2+-channel TRPML1 is PI3,5P2 that is deficient in Fig4-/- cells. We reactivated the TRPML1 channels by application of TRPML1 synthetic ligand, ML-SA1. This treatment reduced the intralysosomal Ca2+-level and rescued abnormal lysosomal storage in Fig4-/- culture cells and ex-vivo dorsal root ganglions (DRG). Furthermore, we found that the suppressed Ca2+-efflux in Fig4-/- culture cells and Fig4-/- mouse brains profoundly down-regulated the expression/activity of dynamin-1, a GTPase known to scissor organelle membranes during fission. This down-regulation made dynamin-1 unavailable for lysosomal fission. Taken together, our study revealed a novel

mechanism explaining abnormal lysosomal storage in FIG4 deficiency. Synthetic ligands of the TRPML1 may become a potential therapy against diseases with FIG4 deficiency.

Disclosures: **J. Li:** None. **J. Zou:** None. **B. Hu:** None. **S. Arpag:** None. **Q. Yang:** None. **A. Hamilton:** None. **Y. Zeng:** None. **C. Vanoye:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.11/W7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NS033202.

Title: Drug discovery using a transgenic model of the slow channel syndrome

Authors: ***C. M. GOMEZ**[1], C. WEI[1], K. ROBINSON[1], B. J. BHATTACHARYYA[2];
[1]Dept Neurol., Univ. Chicago, Chicago, IL; [2]Neurol., Northwestern Univ., Chicago, IL

Abstract: The slow-channel congenital myasthenic syndrome (SCS) is a dominantly inherited disorder of neuromuscular transmission caused by distinct point mutations in the genes encoding the subunits of the muscle acetylcholine receptor (AChR). The SCS is characterized by progressive weakness and fatigability of the voluntary muscles, electrophysiological evidence of an impaired safety factor of neuromuscular transmission, and prolonged endplate currents caused by leaky, mutant AChRs. The leaky AChRs lead to Ca2+ overload degeneration of the post synaptic region. Agents such as the open ion channel blockers, quinidine and fluoxetine, which preferentially block mutant ion channels improve neuromuscular transmission and are neuroprotective in a transgenic model for SCS (mSCS). These two agents are not ideal because of adverse side effects. Here we used our mSCS model (expressing the εL269F mutation) to test new open ion channel agents (amitriptyline, imipramine, memantine and ketanserin) for their capacity to normalize prolonged synaptic currents at the neuromuscular junction and improve neuromuscular transmission presumably by selective blocking of mutant AChRs in SCS mice. Using two microelectrode voltage clamp technique we have recorded spontaneous miniature end plate currents (MEPCs) and evoked end plate currents (EPCs) from excised phrenic nerve diaphragm muscle of εL269F and wild type mice. We found that prolonged MEPC decays (tau) observed in εL269F (19.35±2.8ms compared to wild type 2.4±0.14ms) are significantly reduced in the presence of amitriptyline (IC50~I uM) (10.09±1.9ms; $p<0.05$); we found a similar effect on MEPC decays for each of the other drugs with the following potency range as expressed by IC50 <imipramine (IC50~4uM)<memantine (IC50~6uM) and ketanserin (IC50~12uM). The doses used in this study in the IC50 dose range did not alter either the MEPC frequencies or MEPC amplitudes of εL269F mice, arguing against a presynaptic mechanism of action, and

suggesting that they will be safe for this indication. To further explore the post synaptic mechanism of action of amitriptyline we measured the characteristics of evoked EPCs. After low frequency repetitive stimulation (a rate similar to normal motor neuron firing rate), amitriptyline caused voltage-, as well as concentration-dependent decrease of the decay time constant of the EPCs. These results demonstrate the feasibility of using a transgenic mouse model for SCS to screen for new potential therapeutic agents for this disease. Furthermore, we have detected at least two potentially useful agents to treat mSCS, that are appropriate for preclinical neuroprotective studies.

Disclosures: C.M. Gomez: None. **C. Wei:** None. **K. Robinson:** None. **B.J. Bhattacharyya:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.12/W8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH GRANT10710459

Title: Mutations that suppress structural and functional decline of muscle in a *C. elegans* model of Duchenne muscular dystrophy

Authors: C. BERON[1], A. VIDAL-GADEA[1], J. COHN[1], *J. T. PIERCE-SHIMOMURA[2]; [1]Univ. of Texas at Austin, Austin, TX; [2]Univ. Texas, Austin, Austin, TX

Abstract: Duchenne muscular dystrophy (DMD) is a lethal neurodegenerative disorder characterized by progressive muscular weakness, degeneration, and death. Despite its prominence, a cure has not yet been found for this disease. Previous studies have used animals to model the genetic background of the disease, but have failed to replicate the severity of muscle decline of DMD in humans. The nematode Caenorhabditis elegans has been similarly constrained by these limitations. We developed an assay capable of modulating muscular exertion and recapitulated the human phenotype of DMD in *C. elegans*. Modeling the genetics, locomotor decline, and muscular degeneration characteristic of the disease in *C. elegans*, we performed the first genetic screen to rescue muscle and motor degeneration in an animal without the aid of sensitizing mutations. We isolated several mutations capable of suppressing the locomotor and muscular decline characteristic of DMD. Mapping and cloning of the affected genes will help us identify conserved pathways through which suppressor mutations accomplish their rescue. This will help us produce novel molecular approaches to combat the muscular and locomotor decline characteristic of humans with DMD.

Disclosures: **C. Beron:** None. **A. Vidal-Gadea:** None. **J. Cohn:** None. **J.T. Pierce-Shimomura:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.13/W9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: University of Sydney Bridging Grant APP1087278

Title: Muscle specific kinase autoantibodies: their impact upon the postsynaptic membrane scaffold at the neuromuscular junction

Authors: ***W. D. PHILLIPS**[1], N. GHAZANFARI[1], E. L. T. B. LINSAO[1], S. TRAJANOVSKA[1], S. W. REDDEL[2];
[1]Physiology, Bosch Institute, Univ. of Sydney, Sydney, Australia; [2]Mol. Med. & Neurology, Concord Clin. Sch., Univ. of Sydney, Sydney, Australia

Abstract: A subset of myasthenia gravis (MG) patients possess IgG4 autoantibodies that target Muscle Specific tyrosine Kinase (MuSK) and cause MG when transferred into mice. *In vitro* studies have revealed two mechanisms by which MG patient anti-MuSK could interfere with MuSK at the neuromuscular junction (NMJ). Anti-MuSK disrupted the binding of MuSK to collagen Q (which is thought to tether MuSK in the postsynaptic membrane). Anti-MuSK also blocked the binding of LRP4 to MuSK, thereby inhibiting the activation of MuSK by extracellular neural agrin. Repeated daily injections with MuSK MG IgG for two weeks causes mice to develop myasthenic weakness due to progressive declines in postsynaptic acetylcholine receptor (AChR) density and endplate potential amplitude. Here we have used this mouse passive transfer model to examine the acute *in vivo* effects of MuSK MG IgG at NMJs. Healthy motor endplates in the tibialis anterior muscle displayed strong immunostaining for phosphorylated targets of the MuSK cascade (pSrc and pAChR beta subunit). Phosphorylation of both Src and AChR was reduced 24hours after the first intraperitoneal injection of MuSK MG IgG. In contrast, the efficient targeting of MuSK-EGFP to the endplate was not altered at this early timepoint in the anti-MuSK injection series. These results suggest that suppression of MuSK kinase signaling precedes the loss of MuSK and AChR from the postsynaptic membrane in our mouse model of MuSK MG. Intramuscular injection of adeno associated viral vector was used to force expression of MuSK-EGFP. In healthy mice, MuSK-EGFP targeted to the endplate where it appeared to compete with endogenous MuSK to saturate a postsynaptic MuSK scaffold. In mice receiving a series of daily injections of MuSK MG IgG, endplate immunostaining for MuSK was reduced, but less so in muscles expressing MuSK-EGFP. Endplates expressing MuSK-EGFP also retained most of their original AChR density, when compared to contralateral

control muscles (injected with empty vector). Our results suggest that MuSK autoantibodies cause synaptic disassembly through a process that involves immediate inhibition of MuSK followed by displacement of MuSK from the postsynaptic MuSK scaffold and a slow progressive wastage of endplate AChRs.

Disclosures: W.D. Phillips: None. **N. Ghazanfari:** None. **E.L.T.B. Linsao:** None. **S. Trajanovska:** None. **S.W. Reddel:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.14/W10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ERC 39548

Title: Deficient RNA metabolism as a novel target in neuromuscular disease

Authors: *R. HORVATH;
Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Deficient RNA Metabolism as a Novel Target in Neuromuscular Disease Rita Horvath, Michele Giunta, Veronika Boczonadi, Juliane Müller, Hanns Lochmüller The John Walton Muscular Dystrophy Research Centre, MRC Centre for Neuromuscular Diseases, Institute of Genetic Medicine, Newcastle University The importance of RNA processing in neurodegeneration is highlighted with a rapidly increasing number of human neurogenetic diseases caused by mutations in proteins involved in mRNA metabolism including spinal muscular atrophy (SMA) and pontocerebellar hypoplasias (PCH). A novel mechanism of RNA-associated neurodegeneration has been suggested by the identification of mutations in genes encoding human exosome components. The exosome is a multi-protein complex, required for degradation of AU-rich element (ARE) containing mRNAs, which is an important regulatory step in gene expression. However the human exosome may also regulate gene expression via diverse RNA processing reactions. Mutations in EXOSC3 were reported in pontocerebellar hypoplasia and spinal motor neuron abnormalities (PCH1), and our group recently identified mutations in a novel gene EXOSC8, encoding a core component of the human exosome in children with overlapping symptoms of cerebellar hypoplasia, spinal muscular atrophy and hypomyelination. In a single patient with spinal muscular atrophy (SMA) a potentially disease-causing mutation was detected in RBM7, a co-factor of the exosome complex which binds non-coding PROMoter uPstream Transcripts (PROMPT)s. Despite the complex and central role of the exosome in RNA metabolism in all cell types it is so far not known why and how only specific neuronal cells are affected by exosomal protein deficiency. We investigated how

abnormal RNA metabolism due to defect of exosomal proteins affect gene expression in different human cells *in vitro* and in parallel we studied zebrafish models of exosomal protein deficiencies. Identifying the biochemical pathways of RNA metabolism alterations due to different types of exosome dysfunction may explain why mutations in separate components of the exosome cause different disease presentations, and may identify potential pathways which can be targeted to develop experimental therapies.

Disclosures: R. Horvath: None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.15/W11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NRF Grant 2014-R1A2A1A11052103

KHIDI Grant HI14C1989

Title: Functional and structural evidence of central motor pathway involvement in genetic subtypes of Charcot-Marie-Tooth Disease

Authors: *H. W. LEE[1], M. LEE[1], C.-H. PARK[1], H.-K. CHUNG[1], Y. CHOI[1], J. YOO[2], B.-O. CHOI[3];
[1]Dept. of Neurol., [2]Dept. of Diagnos. Radiology, Ewha Womans Univ. Sch. Med., Seoul, Korea, Republic of; [3]Neurol., Samsung Med. Center, Sungkyunkwan Univ. Sch. of Med., Seoul, Korea, Republic of

Abstract: Background: Charcot-Marie-Tooth (CMT) disease is the clinically and genetically heterogeneous peripheral nervous disorder. There have been a few reports of white matter involvement in some of CMT patients, but detailed pathophysiologic abnormalities in central motor pathways in CMT especially in different genetic subtypes are not fully understood yet. In this study, we investigate the motor cortical excitability and central white matter alterations in CMT patients using transcranial magnetic stimulation (TMS) and Diffusion Tensor Images (DTI). **Methods**: Fifty patients with genetically confirmed CMT (10 CMT1A, 14 CMT1B, 15 CMT2A, 11 CMTX) and 30 control subjects were enrolled. We measured TMS indices for motor cortical excitability including resting motor threshold (RMT), motor evoked potential (MEPs), cortical silent period (CSP), central motor conduction times (CMCT), intracortical inhibition(ICI) and intracortical facilitation (ICF) at different stimulus intensities in upper and lower extremities. DTI was used to analyze the fractional anisotropy (FA), axial (AD), radial (RD) and mean diffusivities (MD). **Results:** In CMT group, RMT increased, CMCT and cortical MEP latencies prolonged, ICI decreased and MEP amplitude decreased in arm and leg muscles

compared to controls. Patients with CMT1B showed more prolonged CMCT and MEP latencies at all TMS stimulus intensities tended to be prolonged than other CMT subtypes. In DTI study, increased FA, AD, MD and reduced RD were noted in bilateral diffuse long white matter tracts in CMT1B group, whereas decreased FA, increased AD, MD and increased RD were noted in more restricted white matter tracts including body of the corpus callosum in CMTX patients. **Conclusion:** Central nervous system involvement in various subtypes of CMT patients was studied using TMS and DTI methods. We found that decreased ICI and delayed CMCT in CMT patients, which means intracortical integrity and central motor pathway alterations in CMT patients. And DTI revealed white matter alterations in mainly CMT2A and CMTX patients. TMS and DTI investigation in CMT patients suggests involvement in central as well as peripheral motor pathways with different pathophysiologic changes in CMT1B and CMTX patients.

Disclosures: H.W. Lee: None. **M. Lee:** None. **C. Park:** None. **H. Chung:** None. **Y. Choi:** None. **J. Yoo:** None. **B. Choi:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.16/W12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant R21NS072675

NIH grant RO1NS054154

Title: Severity of both demyelinating and axonal neuropathy mouse models are modified by genes affecting sodium channels at nodes of Ranvier

Authors: *R. W. BURGESS[1], K. L. SEBURN[2], K. H. MORELLI[3], E. L. SPAULDING[3], D. G. SCHROEDER[2], G. A. COX[2];
[1]The Jackson Lab., Bar Harbor, ME; [2]The Jackson Lab., BAR HARBOR, ME; [3]Graudate Sch. of Biomed. Sci. and Engin., The Univ. of Maine, Orono, ME

Abstract: The genetic basis for the variable severity of Charcot-Marie-Tooth disease (CMT) is being investigated using mouse models of both axonal and demyelinating neuropathy. We identified a mouse strain at the Jackson Laboratory that developed progressive weakness and hind limb dysfunction, leading to paralysis and death by five months of age. A combination of genetic mapping and exome sequencing revealed a double mutation in Sh3tc2 and Neural-glial Related Cell Adhesion Molecule (Nrcam), an Ig-superfamily member cell adhesion molecule involved in development and sodium channel localization at nodes of Ranvier. Independently, the phenotype of Sh3tc2 mutant mice closely resembles previously reported knockout mice

modeling CMT4C, whereas Nrcam mice have only a subtle reduction in nerve conduction velocity and occasional abnormalities in sodium channel (NaV1.6/Scn8a) localization at nodes, but no overt neuropathy phenotype. However, in combination, these mutations result in progressive loss of hind limb function and severe sprouting at neuromuscular junctions, suggesting a failure in action potential propagation in peripheral nerves. This led us to hypothesize that otherwise innocuous or subclinical changes in sodium channel function at nodes (such as those caused by Nrcam mutations) could synergize with mutations affecting the passive propagation of depolarization (the axonal length constant, caused by thin myelin in the Sh3tc2 mutation) to produce a more severe phenotype. We tested this hypothesis by breeding the Nrcam mutation into a Gars mutant background, a mouse model of CMT2D that compromises the axonal length constant by reducing axon diameter without effecting myelination. Loss of Nrcam also exacerbated the Gars phenotype in two different Gars mutant alleles, indicating a synergy of the Nrcam phenotype with axonal as well as demyelinating neuropathy. We validated that the mechanism is due to impaired sodium currents by breeding the Gars mice to Scn8a heterozygous mice, which like Nrcam, have no overt phenotype and only a subtle reduction in nerve conduction velocity. We achieved a very similar enhancement of the Gars phenotype in the Scn8a heterozygous background, supporting our hypothesized mechanism of reduced sodium currents in the Nrcam mutation. Thus, we have established that mutations with minor effects on sodium currents at nodes of Ranvier interact with mutations that reduce the axonal length constant, including both demyelinating and axonal neuropathies, to cause a more severe phenotype. In considering the spectrum of possible modifier loci for CMT, genes encoding node-associated proteins should be considered.

Disclosures: R.W. Burgess: None. **K.L. Seburn:** None. **K.H. Morelli:** None. **E.L. Spaulding:** None. **D.G. Schroeder:** None. **G.A. Cox:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.17/W13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Spanish Ministry of Science and Innovation SAF2009-07063 (to F.P.)

BFU2011-30456-C02-01/BMC (to J.S.)

Generalitat Valenciana Prometeo Programme 2009/059 (to F.P.)

Comunidad de Madrid S2010/BMD-2402 MITOLAB-CM (to J.S.)

Collaborative Joint Project awarded by IRDiRC and funded by ISCIII grant IR11/TREAT-CMT, Instituto de Salud Carlos III (to both F.P. and J.S.)

institutional grant from the Fundación Ramón Areces to the Centro de Biología Molecular Severo Ochoa

FPI fellowship from the Universidad Autonoma de Madrid (to P.G.-S)

Title: Recessive and null GDAP1 mutations associated with Charcot-Marie-Tooth disease reduce Store-operated Ca^{2+} entry (SOCE) and ER Calcium and result in a lower SOCE-stimulation of respiration in intact neural cells

Authors: P. GONZALEZ-SANCHEZ[1,2,3], D. PLA-MARTÍN[5,6], C. B. RUEDA[1,3,7], P. MARTINEZ-VALERO[1,3,7], F. PALAU[4,5,8], *J. SATRUSTEGUI[1,3,2];
[1]Ctr. for Biomed. Res. on Rare Diseases, Dept of Molec Bio, Univ. Autonoma Madrid Ctr. Biol Mol Severo Ochoa (CBMSO), Madrid, Spain; [2]Inst. de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Madrid, Spain; [3]Ctr. de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain; [4]Ctr. de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain; [5]Ctr. de Investigación Príncipe Felipe, Valencia, Spain; [6]Inst. for Genetics, CEDAD Res. Center, Univ. of Cologne, Cologne, Germany; [7]; Inst. de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Madrid, Spain; [8]Hosp. Sant Joan de Déu, Barcelona, Spain

Abstract: Mutations in GDAP1 (Ganglioside-induced differentiation associated protein 1) gene causes Charcot-Marie-Tooth (CMT) neuropathy, the most common inherited neuromuscular disorder. GDAP1 protein is located in the outer mitochondrial membrane and interacts with the vesicle-organelle trafficking proteins suggesting that GDAP1 may participate in the movement of mitochondria. GDAP1 silencing in neuroblastoma SH-SY5Y cells induces abnormal distribution of the mitochondrial network, decreases the contact between mitochondria and endoplasmic reticulum (ER) and reduces 1) Ca inflow through store-operated Ca entry (SOCE) following mobilization of ER-Ca and 2) SOCE-driven Ca entry in mitochondria (1). Any Ca signal causes ATP breakdown by Ca pumps or exchangers in order to restore Ca levels. In addition, Ca regulates oxidative phosphorylation via two different methods: a) Ca entry in mitochondria and activation of mitochondrial dehydrogenases or b) extramitochondrial Ca activation of metabolite transport (2). As SOCE-driven Ca entry in mitochondria is reduced in GDAP1-KD neuroblastoma cells (1), we have investigated mitochondrial respiration during SOCE activation. We find that SOCE drives a marked stimulation of respiration in neuroblastoma cells, which is reduced (by 41%) in the GDAP1 silenced cell line. We have next investigated the effect of different pathological GDAP1 missense mutations in SOCE activity, after transient expression on GDAP1-silenced cells. Recessive mutations in GDAP1 located inside the α-loop (protein-protein interactor domain), such as p.S130C, fail to restore SOCE activity in GDAP1-KD cells. In agreement with this GDAP1 silencing in HEK293T cells decreases SOCE-driven stimulation of respiration and the recessive mutation p.S130C fails to revert the decrease. We now find that cerebellar neurons obtained from Gdap1-knockout mice (3) show reduced Ca inflow through SOCE channels both in soma and neurites compared with WT neurons. Further, in neurites, SOCE-driven Ca entry in mitochondria is abolished. In addition, we have found lower ER-Ca levels in Gdap1-/- neurons, and lower stimulation of respiration by Ca-mobilizing agonists. SOCE-induced stimulation of respiration is also decreased

in Gdap1-/- cerebellar neurons. These findings support a role of altered SOCE and ER-Ca driven Ca uptake and/or signaling in mitochondria in the pathophysiology of GDAP1-related CMT neuropathies, which could impair neuronal respiration and bioenergetics. 1. Pla-Martín D et al., (2013) Neurobiol Dis. 55:140-51. 2.Llorente-Folch I et al., (2013) J Neurosci. 33(35):13957-71. 3. Barneo-Muñoz M et al., (2015) PLoS Genet.11(4):e1005115.

Disclosures: **P. Gonzalez-Sanchez:** None. **D. Pla-Martín:** None. **C.B. Rueda:** None. **P. Martinez-Valero:** None. **F. Palau:** None. **J. Satrustegui:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.18/W14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NS060926

Cure SMA (DID1214)

Title: Electrophysiological and morphological properties of motoneurons in mild and severe mouse models of SMA

Authors: *E. REEDICH[1,2], K. QUINLAN[2], C. J. HECKMAN[2], C. DIDONATO[1];
[1]Stanley Manne Children's Res. Inst., Chicago, IL; [2]Northwestern Univ., Chicago, IL

Abstract: Spinal muscular atrophy (SMA) is a motoneuron disease caused by reduced levels of the survival motor neuron (SMN) protein. Disease severity runs along a spectrum that is generally determined by SMN dosage. Our laboratory has recently generated a mild SMA mouse that exhibits longevity and recapitulates symptomology of mild SMA patients, including motor axon loss and functional deficits. In this mild SMA mouse, the progression of electrophysiological alterations underlying motoneuron loss can be much more clearly delineated than in mouse models of severe SMA, revealing new insights into disease progression and potential treatment windows that precede motoneuron degeneration. Here, we compare the electrophysiological properties of motoneurons from the lower thoracic to upper lumbar spinal cord of neonatal mild and severe SMA mice. Whole-cell patch clamp was performed on medial motoneuron pools in 350μm transverse spinal cord slices from T10 - L2. Properties were analyzed in voltage clamp to study persistent inward currents and in current clamp to study action potential and after-hyperpolarization characteristics as well as frequency-current relationships. We find that while severe SMA motoneurons exhibit overt hyperexcitability, mild SMA motoneurons demonstrate more subtle electrophysiological alterations including increased membrane time constant (τ), decreased cell capacitance (Cm) and increased whole cell input resistance (Rinput). To determine whether smaller motoneuron soma size in mild SMA mice

accounts for these observed electrophysiological abnormalities, three-dimensional anatomical reconstructions were performed on patch-clamped motoneurons. Mild SMA motoneurons tend to have smaller maximal cross sectional areas, soma surface areas, and soma volumes than those of heterozygous control mice, although these values did not reach significance. Currently in progress is a second method to confirm soma size of L1 motoneurons via ventral root backfilling with fluorescent dextrans and those results will be presented. Overall, this work serves to clarify the sequence of electrophysiological changes within motoneurons that precipitates the disease state in SMA.

Disclosures: E. Reedich: None. **K. Quinlan:** None. **C.J. Heckman:** None. **C. DiDonato:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.19/W15

Topic: D.13. Motor Neurons and Muscle

Title: Age-related changes at NMJs and spinal cord synapses of rhesus monkeys

Authors: *N. D. MAXWELL[1], K. VAUGHAN[3,4], M. SZAROWICZ[3,4], R. DE CABO[3], J. A. MATTISON[3], G. VALDEZ[1,2];
[1]VTCRI, Virginia Tech., Roanoke, VA; [2]Virginia Tech., Blacksburg, VA; [3]Translational Gerontology Br., Natl. Inst. on Aging, NIH, Baltimore, MD; [4]SoBran, Inc., Burtonsville, MD

Abstract: While the impact of aging on neuromuscular junctions (NMJs) has been well-documented in mice, it is unclear if the same structural and molecular changes occur in primates. Here, we examined NMJs in the gastrocnemius muscle from adult and aged rhesus monkeys using light microscopy. As in mice, we found significant structural changes in old NMJs that include fragmentation of post-synaptic sites and denervation of muscle fibers. Our analysis also revealed that perisynaptic Schwann cells fail to properly cover aged NMJs. Along with these structural features, we found changes in the level and distribution of NMJ-associated molecules, particularly those associated with the synaptic basal lamina and resident molecules of the post-synaptic apparatus. In addition to the NMJ, we examined α-motor neurons and their synaptic inputs in aged rhesus monkeys. Our analysis revealed a significant reduction in the number of VAChT and VGluT1 positive synapses on the somata of α-motor neurons. The reduced number of synapses in aged spinal cords closely correlates with increasing levels of lipofuscin in the cytoplasm of α-motor neurons. These findings indicate that similar structural and molecular changes occur at the NMJ of rhesus monkeys as previously found in rodents. They also demonstrate the impact of aging on the soma of α-motor neurons and suggest that spinal neuronal circuits are severely impaired in aged monkeys.

Disclosures: **N.D. Maxwell:** None. **K. Vaughan:** None. **M. Szarowicz:** None. **R. de Cabo:** None. **J.A. Mattison:** None. **G. Valdez:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.20/W16

Topic: D.13. Motor Neurons and Muscle

Title: Accelerated motor endplate degeneration in agrin deficient mice after traumatic nerve injury

Authors: D. ZHU[1], J. SU[1], J. JUNG[1], T. ONISHI[1], W. WANG[1], *T. MOZAFFAR[2], R. GUPTA[1];
[1]Orthopaedic Surgery, [2]Neurol., Univ. of California, Irvine, Orange, CA

Abstract: Regenerating axons must navigate over long distances to reinnervate their target muscle; however, muscle atrophy and destabilization of the neuromuscular junction (NMJ) usually occurs before the denervated muscle becomes reinnervated. Agrin has been well characterized as an essential component of NMJ formation during development. We hypothesized that agrin deficient mice would undergo a more rapid and severe atrophy of the motor endplate after denervation with the hopes that agrin delivery could rescue this phenotype. Wildtype and agrin deficient mice were denervated by excision of a 10mm segment of the right sciatic nerve. Wet muscle weight of tibialis anterior muscles were measured and cross sectional areas were evaluated. Protein levels of phosphorylated muscle specific kinase (MuSK) and AChR alpha1 subunits and clustering were evaluated via immunofluorescence. Denervated muscles in agrin deficient mice had a significantly lower wet muscle weight percentage compared to their wildtype counterparts (5.26±0.23 vs 6.20±0.30; p=0.033). Phosphorylated MuSK on the uninjured and injured side was approximately 2-fold and 1.5-fold higher in wildtype mice than in agrin deficient mice at 2 weeks and 4 weeks, respectively. AChR subunits were significantly higher after transection in wildtype than in agrin deficient mice at 4 weeks (1±0.30 vs 1.94±0.24; p=0.032). After denervation, pixel density and AChR area decreased more severely in agrin deficient mice than in wild type mice by 8 weeks. Furthermore, morphological assessment of AChR revealed a rapid shift in the receptor morphology towards plaque-like profiles in agrin deficient mice when compared to wildtype mice at both 2 and 8 weeks. Heterozygous agrin deficient mice have depleted agrin levels at their denervated motor endplates. After traumatic nerve injury, these mice exhibit decreased phosphorylation of MuSK and accelerated disassembly of AChRs when compared to their wildtype counterparts. Most importantly, this data presents novel data to support further study of agrin and its essential role in adult NMJ stability and maintenance after injury.

Disclosures: **D. Zhu:** None. **J. Su:** None. **J. Jung:** None. **T. Onishi:** None. **W. Wang:** None. **T. Mozaffar:** None. **R. Gupta:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.21/W17

Topic: D.13. Motor Neurons and Muscle

Title: Functional evaluation of the effects of aging and genetic manipulations on motor behavior

Authors: ***B. MAREIN**[1], N. LOZIER[2], S. DE LACALLE[2];
[1]Col. of Arts & Sci., Athens, OH; [2]Biomed. Sci., Ohio Univ., Athens, OH

Abstract: Finding ways to slow and reverse muscle wasting remains an urgent task for an increasingly aging society, and myostatin (Mstn) has been presented as an attractive therapeutic candidate for the treatment of muscle wasting because its inactivation results in muscle hypertrophy. Our laboratory has started to analyze changes in motor behavior with aging, using two different animal models. First, we investigated motor function in an aged group (19 months of age, n=7) vs. a young adult group (6 months of age, n=8) of C57/BL6 male mice. While hind limb grip strength scores were not significantly different when normalized to body weight, the young adult group significantly outperformed the aged group on the rotarod test of endurance and balance. Then we set to investigate whether Mstn signaling plays a role in age related muscle function decline. We have developed a mouse model in which a critical segment of the Mstn gene (exon 3) is flanked by loxP (floxed) and also contains a Doxycycline(DOX)-inducible Cre recombinase transgene, dubbed DOX-inducible MSTN KO mice. Treatment with DOX renders the Mstn gene non-functional by excising the DNA flanked by the LoxP sequences. As expected, following 14 weeks of DOX administration in the chow, we found a significant increase in triceps surae weights of the DOX treated (7 months of age, n=11) vs. the untreated male mice (7 months of age, n=9), resulting in significantly higher absolute hind limb grip scores in treated mice, but there was no significant difference between hind limb grip scores when normalized to body weight. Biceps and triceps brachii muscles, though not significant, were slightly larger in DOX treated mice, but there was no difference between the groups in absolute forelimb grip strength or when normalized to body weight. DOX treated mice exhibited significantly larger masseters, but there was no significant difference in absolute bite force or when normalized to masseter weight. Likewise, there was no significant difference in time spent on the rotarod. These results suggest that Mstn inhibition in the young adult, while increasing muscle size, does not positively affect muscle function. Tests of an aging group of DOX-inducible MSTN KO mice are currently underway, and may enhance our understanding of the role of Mstn in the behavioral correlates of muscle aging.

Disclosures: **B. Marein:** None. **N. Lozier:** None. **S. de Lacalle:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.22/W18

Topic: D.13. Motor Neurons and Muscle

Support: Adelson Program in Neural Repair and Rehabilitation

Title: Identifying new targets to promote muscle regeneration

Authors: ***J. T. EHMSEN**[1], R. MI[1], G. COPPOLA[2], A. HOKE[1];
[1]Johns Hopkins, Baltimore, MD; [2]UCLA, Los Angeles, CA

Abstract: Skeletal muscle atrophy is a loss of muscle mass and corresponding loss of function that occurs in response to diverse stimuli including disuse/immobility, hyperthyroidism, diabetes, glucocorticoid treatment, cancer, aging, and denervation. Denervation is a significant contributor to muscle wasting in trauma, degenerative diseases, and age-associated sarcopenia in humans. Muscle satellite cells possess the unique capacity to proliferate and regenerate in settings of muscle injury, but regenerative capacity becomes depleted during chronic denervation. Applying microarray and RNA-Seq approaches, we are investigating mechanistic features of atrophy during acute and chronic denervation in both muscle and muscle satellite cells of mouse gastrocnemius using a tibial nerve denervation model, with the intention of identifying novel targets for minimizing atrophic changes and/or sustaining or enhancing muscle satellite cell proliferative and regenerative capacity.

Disclosures: **J.T. Ehmsen:** None. **R. Mi:** None. **G. Coppola:** None. **A. Hoke:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.23/W19

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Farber Family Foundation Grant F96401

Title: Mutant FUS mediated impairments in mRNA trafficking and protein translation at NMJ

Authors: *K. KRISHNAMURTHY[1], N. ALAMI[3], J. P. TAYLOR[4], D. TROTTI[2], P. PASINELLI[2];
[1]NEUROSCIENCE, [2]Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [3]Stanford Univ., California, CA; [4]Cell & Mol. Biol., St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset rapidly progressing neurodegenerative disease that leads to fatal paralysis. About 10% of ALS cases are familial (fALS), and are associated with mutations in one or another of several genes including the DNA/RNA binding protein Fused in Sarcoma (FUS). Like for other forms of ALS, the hallmark of FUS-ALS is destruction of the neuromuscular junction (NMJ). Accordingly, we showed that NMJ defects, and specifically, defects in synapse structure and function precede motor neuron degeneration in a *Drosophila* model of FUS-ALS (Shahidullah et al., 2013). We also showed mutant FUS reduces levels of the presynaptic proteins SV2 and synaptophysin in motor neurons without causing significant changes in their mRNA transcripts. At the functional level,mutant FUS impairs neurotransmitter release (Krishnamurthy et al., 696.04/F5 SFN 2014). Here we study the mechanism(s) by which NMJs become dysfunctional in mutant FUS models. We show that expression of mutant FUS in NSC-34 cells and motor neurons results in the up-regulation of ER stress marker phospho-eIF2α, and possible impairment of the axonal trafficking and translational machinery

Disclosures: K. Krishnamurthy: None. **N. Alami:** None. **J.P. Taylor:** None. **D. Trotti:** None. **P. Pasinelli:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.24/W20

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR Grant 14137

ALS Canada Bernice-Ramsay

Title: Persistent motor-unit specific synaptic alterations at the neuromuscular junction in the SOD1G37R mice

Authors: *E. TREMBLAY, É. MARTINEAU, R. ROBITAILLE;
Univ. De Montréal, Montreal, QC, Canada

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal late-onset neurodegenerative disease characterized by the progressive loss of motoneurons. Denervation of the neuromuscular junction (NMJ) is an early pathological event in various ALS models. Motor units (MU) appear unequally

susceptible to denervation, the fast fatigable (FF) MU being the most vulnerable and the slow (S) MU the most resistant. While previous studies in several ALS models have consistently reported alterations in synaptic transmission, their findings have been contradictory. Interestingly, the MU types were not taken into account in these studies, which could explain these discrepancies. We hypothesized that the MU selective vulnerabilities observed in ALS will be associated with MU-specific NMJ alterations throughout the disease course. We studied synaptic transmission and plasticity of different types of MU in the fast-twitch Extensor Digitorum Longus (EDL; fast fatigable (FF) MU) and the slow-twitch Soleus (SOL; slow (S) and fast fatigue resistant (FR) MU) of the SOD1 mice and their WT littermates. MU types were identified using immunohistochemical labelling of the respective myosine heavy chains. At a presymptomatic stage (P160), while no morphological alterations of NMJs were seen in both muscles, synaptic activity was altered in a MU-specific manner in SOD1 mice. S and FR MU had an increased mEPP frequency whereas no difference was seen for FF MU in SOD1 compared to WT. Similar changes were observed in evoked activity where FF MU from SOD1 mice showed a decrease in EPP amplitude and quantal content whereas S MU showed a higher quantal content. FR MU of SOD1 showed no difference in evoked activity compared to WT. Long-term synaptic plasticity was significantly reduced in the FF MU of SOD1. At preonset (P400), various morphological alterations were seen in the mutant mice, most strikingly in the EDL muscle, including denervation, partial innervation and nerve sprouting. Evoked activity was altered in a similar way as was observed at P160 for all MU types. In contrast, spontaneous activity was now reduced in the mutant FF MUs, whereas mEPP frequencies of S and FR MU were no longer different compared to WT. Interestingly, the increase in quantal content of the S MU was almost absent at disease onset. Surprisingly, paired-pulse facilitation was not altered in any MU type and at any age despite changes in synaptic strength. Taken together, these results reveal that NMJ function is differentially altered according to MU susceptibility in ALS. This study provides insights for a better understanding of NMJ physiology during the illness that is crucial to the development of a proper NMJ-targeted treatment in ALS.

Disclosures: **E. Tremblay:** None. **É. Martineau:** None. **R. Robitaille:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.25/W21

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Wellcome Trust

NMDA

Title: Muscle dysfunction occurs prior to symptom onset and motor neuron degeneration in a mouse model of Spinal Bulbar Muscular Atrophy

Authors: *L. V. ANNAN, A. L. GRAY, B. MALIK, L. GREENSMITH;
Sobell Dept. of Motor Neurosci. and Movement Disorders, UCL Inst. of Neurol., London, United Kingdom

Abstract: Spinal and bulbar muscular atrophy (SBMA), otherwise known as Kennedy's disease, is an X-linked, late-onset progressive neurodegenerative disease, which predominantly affects males. SBMA is characterised by the selective loss of spinal and bulbar motor neurons and progressive muscle weakness. The disease is caused by an expansion in the CAG repeat in the androgen receptor (AR) gene which encodes a polyglutamine (poly-Q) tract in the mature protein (La Spada et al., 1991, Fischbeck, 2001). The polymorphic trinucleotide CAG repeat normally ranges from 9 to 36, but an expansion of greater than 38 repeats results in disease. Although the underlying pathophysiology of the disease remains largely unknown, it appears to be related to abnormal accumulation of the pathogenic androgen receptor protein within the nucleus. Although widely considered a neurodegenerative disease affecting motor neurons, emerging evidence suggests that SBMA may involve a primary muscle deficit. In this study we examined this possibility by systematically characterising hindlimb muscle function and histopathology at different stages of disease progression in the AR100 mouse model of SBMA, which recapitulate the key features of the human disease. Our results show that muscle atrophy, denervation and mitochondrial dysfunction are evident during early stages of disease in AR100 mice, prior to any loss of motor neurons. Using *in vivo* physiological recordings in anaesthetised mice, we detected a significant reduction in muscle force by 6 months of age. We also observed a reduction in motor unit survival at 6 months, indicative of muscle denervation, although motor neuron death was only detected at 18 months. Furthermore, the muscle deficits were much more pronounced in the fast twitch muscle tibialis anterior (TA) than the slow twitch soleus muscle, which remained largely unaffected, even at 18 months. The deficits observed in TA were accompanied by a change in the histochemical properties of the muscle fibres, which showed an increase in oxidative capacity, as revealed by staining for the mitochondrial respiratory enzyme, succinate dehydrogenase. Taken together, these results support the proposal that skeletal muscle is a primary target of pathology in SBMA, which may be important for the development of therapeutic strategies for SBMA. References FISCHBECK, K. H. 2001. Polyglutamine expansion neurodegenerative disease. *Brain Res Bull,* 56**,** 161-3. LA SPADA, A. R., WILSON, E. M., LUBAHN, D. B., HARDING, A. E. & FISCHBECK, K. H. 1991. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature,* 352**,** 77-9.

Disclosures: L.V. Annan: None. **A.L. Gray:** None. **B. Malik:** None. **L. Greensmith:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.26/W22

Topic: D.13. Motor Neurons and Muscle

Title: Fine motor kinematic analysis in the MDX mouse model of Duchenne muscular dystrophy a study of chronically exercised versus non-exercised MDX mice

Authors: P. J. SWEENEY[1], T. BRAGGE[1], *T.-K. STENIUS[1], A. NURMI[1], T. HEIKKINEN[1], T. AHTONIEMI[1], D. WELLS[2];
[1]Charles River Discovery Services, Kuopio, Finland; [2]Royal Vet. Col., London, United Kingdom

Abstract: Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene that is on the X chromosome; lack of dystrophin causes a progressive muscle necrosis which leads to progressive decrease in mobility for those suffering from the disease. There is currently no cure for this disease and those suffering from DMD generally die in their twenties or early thirties. The MDX mouse (a murine model with a mutation causing dystrophin deficiency) has been used to study the efficacy of drugs aimed at DMD especially beyond the 'critical period' of 3 to 7 weeks when MDX muscles show significant spontaneous signs of damage under histopathological examination as well as with magnetic resonance imaging (MRI). While there are many 'traditional' assays employed to assess the degree of muscle weakness at various ages it has become apparent that MDX mice do not develop clear muscle weakness or manifest observable changes in gait or motility at ages older than the 'critical period'. Moreover, the clinical utility of these traditional assays has been questioned and this has increased the need to create novel assays that are more sensitive to subtle deficits in motor function In this study we employed high speed kinematic analysis with a unique algorithm in order to track, record and analyze the fine motor movement of MDX mice that had been chronically exercised versus MDX mice that had not been exercised. In this analysis the relationships between specific body parts are measured with a high speed camera as the mouse walks through a mirrored chamber. The movement of the animal is recorded from below and from the right and left in order to assess multiple body parts from three angles allowing for a comprehensive analysis of motor function. The resulting analysis by the algorithm provides a view of subtle changes in gait and a principle component analysis that allows the operator to see the relationships between certain key anatomical areas during a single or multiple strides. In addition, by breaking the behavior into components, we have been able to provide a much richer characterization of the motor deficits that are apparent in the MDX animal model. The results show that there are no clearly significant differences in principle components in the unexercised MDX mouse relative to the WT mouse at all ages but, more importantly, there are significant changes between exercised and non exercised MDX mice at all ages. These data provide further support for the use of the high precision kinematic assay as a tool for assessing motor function in more sensitive and comprehensive manner.

Disclosures: **P.J. Sweeney:** None. **T. Bragge:** None. **T. Stenius:** None. **A. Nurmi:** None. **T. Heikkinen:** None. **T. Ahtoniemi:** None. **D. Wells:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.27/W23

Topic: D.13. Motor Neurons and Muscle

Title: Comparison of longitudinal (12 month) profile of chronically exercised vs unexercised MDX mouse model of Duchenne muscular dystrophy (DMD) using high field (11.7 Tesla) MRI & 1H-MRS

Authors: *P. J. SWEENEY[1], T. AHTONIEMI[1], J. PUOLIVÄLI[1], K. LEHTIMÄKÍ[1], P. KARHUNEN[1], T. HEIKKINEN[1], A. NURMI[1], D. WELLS[2];
[1]Charles River Discovery Services, Kuopio, Finland; [2]Royal Vet. Col., London, United Kingdom

Abstract: Duchenne muscular dystrophy (DMD) is an X-linked and lethal muscle wasting disease and considered the most severe form of muscular dystrophy. There currently exists no treatment and most sufferers die between the second and fourth decade of life. The MDX mouse model has been extensively employed as a tool to study drug efficacy in DMD but there does not currently exist an extensive longitudinal profile utilizing non-invasive imaging with T2 MRI and 1H-MRS. In addition, imaging has not been used to compare animals with a phenotype that has been exacerbated in order to create a progressive muscle damage that is more severe with those, aged matched, that have not been chronically exercised and in so doing attempt to gauge a wider therapeutic window. In this study we employed an 11.7 T small animal Bruker MRI in order to compare the temporal profiles of exercised vs unexercised MDX mice over a 12 month period. Altogether 25 male MDX mice (mice with the mutation characteristic of DMD) and 25 male wild-type (WT) mice were profiled for baseline MRI/MRS and plasma creatine kinase (pCk) starting at 7 weeks of age and again at the 3 months, 6 months, 9 months and 12 months time points. At the age of approximately 7 weeks 15 MDX and 15 WT mice were subjected to a chronic exercise regime. These were later compared with aged matched controls (non-exercised) MDX and WT mice. The exercised mice were subjected to a chronic regime that consisted of running and avg of 3 times per week 20 minutes at a max speed of 14 meters/min on an inclined treadmill in an attempt to exacerbate muscle damage associated with the underlying disease pathology. The longitudinal development of this pathology was examined and compared using T2 MRI and 1H-MRS in order to obtain a morphological and metabolic profile from the gastrocnemius muscle and the tibialis posterior muscle of all mice at various ages. The results indicate that the temporal metabolic and morphological profile is significantly different between those MDX mice that are chronically exercised beyond the “critical period” mice and those MDX mice that are not subjected to the exercise regime. This information shows that a widened therapeutic window is apparent and can be used to study these mice for longer periods of time. In addition, this information indicates that high field MRI and MRS are useful tools for the quantification of muscle damage in the MDX mouse.

Disclosures: **P.J. Sweeney:** None. **T. Ahtoniemi:** None. **J. Puoliväli:** None. **K. Lehtimäkí:** None. **P. Karhunen:** None. **T. Heikkinen:** None. **A. Nurmi:** None. **D. Wells:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.28/W24

Topic: D.13. Motor Neurons and Muscle

Support: GH Grant CONACyT 377111

PFA C-703/2013 (UV)

Title: Evaluation of supplementation of DHA (docosahexaenoic acid) in the electromyographic activity of mandibular tremors in Parkinson's rat model

Authors: *G. HERRERA MEZA[1], R. OLIART ROS[2], L. I. GARCÍA[3], A. MARTÍNEZ-CHACÓN[4], S. HERRERA MEZA[5];
[1]Unidad De Investigaciones Alimentarias (UNIDA) Ins, Xalapa, Mexico; [2]Unidad de Investigación y Desarrollo en Alimentos, Inst. Tecnológico de Veracruz, Veracruz, Mexico; [3]Ctr. de Investigaciones Cerebrales, [4]Inst. de Neuroetología, [5]Inst. de Investigaciones Psicológicas, Univ. Veracruzana, Xalapa, Mexico

Abstract: The absence of polyunsaturated fatty acids (omega-3 PUFAs) specifically DHA (docosahexaenoic acid) is related neuropathology as Parkinson's disease. Furthermore DHA appears to have neuroprotective and restorative effect, which has caused broad interest to know the effects of DHA in brain function. However, little is known of their involvement in parkinsonism. The aim of study was to describe electromyography mandibular tremors induced by subchronic treatment with haloperidol in rats previously supplemented with DHA. Rats were previously subjected to chronic supplementation of 6 weeks (21-63 days old) the esophageal route with DHA, and then were induced by subchronic administration mandibular tremors (14 days) of haloperidol (Ip) in a model of parkinsonism. The amplitude of the EMG activity and the number of bursts per second was analyzed using generalized linear models (GLM) with a design of fixed factors and nested with pseudoreplication. The supply of DHA did not fully compensate the motor deficits induced by haloperidol and does not eliminate the tremors, but there were changes electromyographic (EMG) activity in the basal and during temporal mandibular muscle tremors. In contrast, DHA was effective to reduce the animal's immobility time and reduced latency in a spontaneous exploration open field test. Suggesting neuroprotection on difficulty initiating movements, acinesia. The beneficial effects of DHA were evident in the electromyographic activity of animals which were supplemented with DHA only about rats supplied with haloperidol.

Disclosures: **G. Herrera Meza:** None. **R. Oliart Ros:** None. **L.I. García:** None. **A. Martínez-Chacón:** None. **S. Herrera Meza:** None.

Poster

339. Neuromuscular Disorders

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 339.29/W25

Topic: D.13. Motor Neurons and Muscle

Support: Swank Foundation

Nemours Foundation

Title: Correlation of neuromotor apparatus disruption and required dosage of neuromuscular blocking agent during anesthesia in children with cerebral palsy

Authors: ***S. K. YEAGER**, K. G. ROBINSON, R. E. AKINS, Jr;
Nemours Alfred I. Dupont Hosp. For Children, Wilmington, DE

Abstract: The cerebral palsies (CP) are static encephalopathies arising during fetal or early post-natal life and characterized by disordered movement. Previous reports indicate that children with CP are resistant to non-depolarizing neuromuscular blocking agents (ND-NMBAs) during surgery. ND-NMBAs competitively inhibit nicotinic acetylcholine receptors (AChRs) at neuromuscular junctions (NMJs), and differences in dosing can indicate disruption of NMJ components. Patients with denervating trauma, burns, or injury have elevated expression of the non-junctional, fetal AChR isoform resulting in resistance to ND-NMBAs. Gene expression studies, however, indicate that fetal AChR subunits are not present in CP patients, and an alternative explanation is needed to account for the ND-NMBA resistance in CP patients. Microanatomic and ultrastructuctural studies indicate that patients with CP have disorganized NMJs, and we suspect that ND-NMBA resistance correlates with the degree of NMJ disruption. To test this idea, pediatric patients were enrolled in an IRB-approved study after obtaining informed consent and assent for muscle sample collection during surgery. Total doses of rocuronium (ROC), a frequently used ND-NMBA in pediatric surgery, were derived from the patient's surgical record. The standard ROC dosage in children is 0.6mg/kg, but additional ROC may be used to achieve the desired muscle relaxant effect as assessed by train-of-four monitoring. Biopsies of erector spinae, extra-ocular, cremaster, vastus lateralis, and rectus femoris muscles were obtained from surgeries then snap-frozen in liquid nitrogen chilled isopentane, sectioned, and stained for synaptic acetylcholinesterase (AChE), laminin β2, and nicotinic acetylcholine receptor (AChR). The degree of NMJ disruption within the sample was determined using a validated score based on the microanatomic distribution of NMJ components determined based on fluorescently tagged probes. Dysmorphism was quantified as the degree of

non-co-localization of these components using fluorescence microscopy and a co-localization algorithm. The greatest NMJ dysmorphism related to spastic CP was staining of AChE relative to laminin β2 ($p < 0.001$). ROC doses for the CP and non-CP groups were also significantly different ($p=0.002$) with the non-CP patients requiring 0.66 + 0.07 mg per kg (mean + SD; n=20) and the CP patients requiring 1.38 + 0.80 mg per kg (n=20). Specific correlations between ROC dosage and NMJ disruption score for each patient are being carried out. The present findings indicate that children with CP have disrupted NMJs and a higher requirement for ROC.

Disclosures: **S.K. Yeager:** None. **K.G. Robinson:** None. **R.E. Akins:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.01/W26

Topic: D.15. Basal Ganglia

Support: PIUNA 14-15

"UTE Project CIMA"

Title: Quantitative study of pallidotegmental projections contacting cholinergic, calbindin-, and calretinin- immunoreactive neurons in the rat pedunculopontine and laterodorsal tegmental nuclei

Authors: S. MONGIA[1], E. LUQUIN[1], *E. MENGUAL[2];
[1]Anatomía, Fac Med, Univ. de Navarra, Pamplona, Spain; [2]Depto. Anatomía, Fac Med, Univ. De Navarra, Pamplona, Spain

Abstract: The pedunculopontine tegmental nucleus (PPTg) is a brainstem target of basal ganglia output particularly implicated in gait and posture. Deep brain stimulation of the PPTg in Parkinson's disease patients has provided partial alleviation of gait disorders, although the mechanism of action of this therapy is not fully understood. This could be partly due to the lack of anatomical data about the fine circuitry in PPTg and the associated laterodorsal tegmental nucleus (LDTg). Specifically, the phenotype of the target cells of pallidotegmental projections is not known yet. PPTg and LDTg comprise intermingled cholinergic, GABAergic and glutamatergic cells; in addition, glutamatergic and GABAergic cells generally coexpress a calcium-binding protein, either calretinin (CR) or calbindin (CalB). As a first step to phenotypically characterize the cell targets of pallidotegmental projections, injections of the anterograde tracer biotinylated dextran amine (BDA) were carried out in the rat ventral pallidum and entopeduncular nucleus, followed by triple fluorescence labeling to visualize the BDA-labeled fibers, the cholinergic - choline acetyltransferase-immunoreactive (ChAT-ir) - neurons, and either CR-, (CR-ir) or CalB-immunoreactive (CalB-ir) neurons in single coronal sections.

Confocal images were analyzed using the analysis software- Fiji - a plugin of ImageJ - which reveals 'slice by slice' colocalization, that is, the positive voxels within a given area where colocalization is detected throughout the stack, facilitating the quantification of potential BDA contacts with defined cell populations. Putative synaptic contacts were observed with each of the three populations analyzed, and the mean percentage of contacts received by each was very similar in the PPTg and LDTg (CR-ir: 46% and 47% of contacts, respectively; ChAT-ir: 32% and 34%; CalB-ir: 22% and 19%). These results indicate that: 1) almost 1/3 of contacts are made on cholinergic neurons, in contrast to previous estimations; this anatomical evidence supports a significant role of PPTg and LDTg cholinergic cells in basal ganglia circuitry, consistent with the pathological results observed in Parkinson's disease post-mortem tissue; 2) pallidotegmental fibers preferentially target CR-ir neurons in both PPTg and LDTg; and 3) over 2/3 of contacts are made with either CR- or CalB-ir neurons: as previous studies have shown that 2/3 of either subpopulation are glutamatergic, the present data suggest that the largest postsynaptic target of pallidotegmental fibers in PPTg and LDTg is glutamatergic.

Disclosures: **S. Mongia:** None. **E. Luquin:** None. **E. Mengual:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.02/W27

Topic: D.15. Basal Ganglia

Support: Swedish Research Council: VR-M-K2013-62X-03026

VR-NT 621-2007-6049

Human Brain Project

Karolinska Institutet's Research Funds

Title: Direct dopaminergic projections from the SNc modulate tectal motor responses

Authors: *J. PÉREZ-FERNANDEZ, A. KARDAMAKIS, B. ROBERTSON, S. GRILLNER; Karolinska Inst., Stockholm, Sweden

Abstract: In the lamprey, as in mammals, dopamine plays a key role in movement control by modulating the excitability of projection neurons in the striatum. The direct "go" pathway neurons express the dopamine D1 receptor and mediate a net facilitation of motor actions desinhibiting the pallidal GABAergic output neurons, whereas the indirect "no go" pathway, through the D2 subtype, mediates motor suppression. The dopaminergic modulation of the striatum derives from the nucleus of the posterior tuberculum, the homologous region of the mammalian substantia nigra pars compacta (SNc). It shows the same connectivity with the other

basal ganglia subnuclei observed in mammals, and receives pallial (cortex in mammals) and tectal input as the evolutionary basis for salience/novelty detection. The importance of this dopaminergic innervation in the lamprey is reflected in the fact that, when depleted, it gives rise to a marked hypokinesia, as in Parkinson's disease. One important feature is that the SNc in lamprey, as in mammals, sends direct dopaminergic projections to motor command centres, including the diencephalic and mesencephalic motor regions and the output layer of the optic tectum. The nigral dopaminergic control of motor responses is thus likely to be more complex than generally assumed, involving additional pathways to the widely studied striatal projection. Here, we explore how dopamine modulates motor responses in the optic tectum, the homologous region of the mammalian superior colliculus. Tectum shows in all vertebrates, including the lamprey, similar features, with a laminated structure, controlling eye, orienting and evasive trunk movements. Tracer injections combined with immunocytochemistry show that dopaminergic fibers from the SNc innervate the deep layer premotor cells, which express D1 and D2 receptors. Patch-clamp recordings show that D1 and D2 cells are separated populations, and dopamine increases the excitability of D1 cells, whereas it decreases the excitability of D2 cells, so that dopaminergic modulation changes their responsivness to sensory inputs reaching the optic tectum. Electromyography (EMG) recordings in the eye muscles show that motor responses elicited by retinal stimulation can be modulated by locally injected dopamine agonists in the optic tectum. Our results indicate that dopamine directly modulates motor responses in premotor regions and, given the high degree of conservation of the basal ganglia and the presence of direct dopaminergic projections from the SNc to the superior colliculus in rats, this previously unexplored mechanism is likely to also be present in higher vertebrates including primates.

Disclosures: J. Pérez-Fernandez: None. **A. Kardamakis:** None. **B. Robertson:** None. **S. Grillner:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.03/W28

Topic: D.15. Basal Ganglia

Support: NIH NINDS grant 2R37 NS041280

NIH NINDS grant P50 NS047085

Title: Optogenetic interrogation of the Parkinsonian subthalamic nucleus-external globus pallidus network during cortical slow-wave activity

Authors: *R. F. KOVALESKI[1], J. W. CALLAHAN[2], M. D. BEVAN[2];
[2]Physiol., [1]Northwestern Univ., Chicago, IL

Abstract: The reciprocally connected subthalamic nucleus (STN)-external globus pallidus (GPe) network is a key component of the movement-suppressing hyperdirect and indirect pathways of the cortico-basal ganglia-thalamo-cortical circuit. The symptomatic expression of experimental and idiopathic Parkinson's disease (PD) is associated with the emergence of abnormally persistent, widespread, correlated, rhythmic (1-30 Hz) cortico-basal ganglia-thalamo-cortical circuit activity. Because the STN-GPe network is a candidate amplifier of abnormal activity throughout this circuit, we applied optogenetic silencing and concurrent multisite recording in control and unilateral 6-hydroxydopamine (6-OHDA)-treated mice to determine how parkinsonian STN-GPe network activity is generated. In control mice under urethane anesthesia, STN neuron firing was largely entrained to the active component of the slow cortical oscillation, whereas GPe neurons discharged in a relatively tonic manner. In 6-OHDA-treated mice, the phasic activity of STN neurons increased (by 75%) and the proportion of GPe neurons that exhibited firing that was correlated with cortical slow-wave activity (SWA) increased (control = 61%; 6-OHDA = 85%), with neurons firing in anti-phase (control = 14%; 6-OHDA = 75%), in-phase (control = 47%; 6-OHDA = 10%), or with no significant phase preference (control = 39%; 6-OHDA = 15%). Cre-dependent expression of ArchT-eGFP in PV+ GPe neurons confirmed their prototypic nature, i.e. their extensive projection to the STN. In 6-OHDA-treated mice, optogenetic silencing revealed that PV+ GPe neurons discharged in anti-phase to SWA (52%) or tonically (48%). Optogenetic inhibition of PV+ GPe neurons increased the firing of STN (control = 92%; 6-OHDA = 102%) and PV- GPe neurons (control = 48%; 6-OHDA = 56%). Optogenetic inhibition of STN neurons decreased the firing of GPe neurons in both control and 6-OHDA-treated mice (control = 16%; 6-OHDA = 32%) and promoted anti-phasic discharge relative to SWA in the latter. In dopamine-depleted mice, optogenetic inhibition of D2-expressing striatal projection neurons (D2-SPNs) disinhibited GPe neurons and eliminated anti-phasic firing relative to SWA. Together these data suggest that following loss of dopamine, hyperexcitable D2-SPNs excessively inhibit prototypical PV+ GPe-STN neurons leading to disinhibition of STN and PV- GPe neurons and excessive cortical patterning of the STN-GPe network.

Disclosures: **R.F. Kovaleski:** None. **J.W. Callahan:** None. **M.D. Bevan:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.04/W29

Topic: D.15. Basal Ganglia

Support: CIHR grant MOP-115008

NSERC grant 401848-2011

FRQS doctoral fellowship 14D 29441

Title: The dopamine innervation of the primate pallidum: A comparison between the internal and external segments

Authors: *L. EID, M. PARENT;
Psychiat & Neurosci Dept, Univ. Laval, Quebec, QC, Canada

Abstract: The internal (GPi) and external (GPe) segments of the pallidum are innervated by dopamine (DA) axons that arise mainly from the substantia nigra pars compacta. The present light and electron microscopic study provides a detailed description of this innervation in squirrel monkeys (*Saimiri sciureus*), by using an antibody raised against tyrosine hydroxylase (TH), the DA synthesizing enzyme. At the light microscopic level, our data gathered with an unbiased stereological approach indicate a similar density of TH+ axon varicosities in the GPi ($0.17 \pm 0.01 \times 10^6$ axon varicosities/mm^3 of tissue) and the GPe ($0.19 \pm 0.02 \times 10^6/mm^3$), the anterior sector of the GPi ($0.23 \pm 0.01 \times 10^6/mm^3$) and the GPe ($0.24 \pm 0.01 \times 10^6/mm^3$) being twice as much densely innervated than their posterior counterparts (0.10 ± 0.01 and $0.14 \pm 0.02 \times 10^6/mm^3$, respectively). A decreasing gradient of innervation also occurs along the dorsoventral axis of both pallidal segments (0.21 ± 0.01 vs. $0.14 \pm 0.01 \times 10^6/mm^3$ in the GPi and 0.22 ± 0.01 vs. $0.17 \pm 0.01 \times 10^6/mm^3$ in the GPe) and along the mediolateral axis of the GPe (0.29 ± 0.02 vs. $0.12 \pm 0.01 \times 10^6/mm^3$). When considering the neuronal density of the GPi ($2.69 \pm 0.18 \times 10^3$ neurons/mm^3) and the GPe ($3.47 \pm 0.15 \times 10^3$ neurons/mm^3), our data indicate that the ratio of TH+ axon varicosities/pallidal neuron is two times higher in the GPi (68 ± 15) than in the GPe (28 ± 3). Our electron microscopic analysis reveals that the TH+ axon varicosities in the GPi are significantly larger than those in the GPe. Moreover, the TH+ axon varicosities observed in the GPi are significantly larger than unlabeled profiles selected at random. All TH+ axon varicosities detected in the GPi and GPe are filled with synaptic vesicles, but very few are engaged in synaptic contacts ($15 \pm 4\%$ in the GPi and $17 \pm 3\%$ in the GPe). These rare synaptic contacts are of the symmetrical and asymmetrical type in equal proportions. Large myelinated TH+ axons also occur in both pallidal segments. Our ultrastructural analysis shows that DA axons can either directly modulate pallidal neurons through synaptic contacts, or indirectly through volume transmission. This diffuse mode of release may allow DA to bind on postsynaptic receptors located on pallidal neurons, as well as on presynaptic receptors located on incoming pallidal afferents. Our findings provide a detailed picture of the regional distribution and fine morphological features of the DA innervation of the primate pallidum. They indicate that, in addition to the action they exert at the striatal level, nigral DA neurons have a direct access to pallidal neurons and their afferents, including neurons of the GPi, which are major output elements of the basal ganglia.

Disclosures: L. Eid: None. **M. Parent:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.05/W30

Topic: D.15. Basal Ganglia

Support: PIUNA 2014-2015

Title: Distribution of GABA-A receptor alpha 1 subunit and GABA-B receptor R2 subunit immunoreactivities in the pedunculopontine and laterodorsal tegmental nuclei in the rat

Authors: *E. LUQUIN, B. PATERNAIN, E. MENGUAL;
Dpto. Anatomia, Fac. De Medicina, Univ. De Navarra, Pamplona, Spain

Abstract: The pedunculopontine tegmental nucleus (PPTg) is a brainstem target of basal ganglia output particularly implicated in gait and posture. Deep brain stimulation of the PPTg in Parkinson's disease patients has provided partial alleviation of gait disorders, although the mechanism of action of this therapy is not fully understood. This could be partly due to the lack of anatomical data about the fine circuitry in PPTg and the associated laterodorsal tegmental nucleus (LDTg). The output projections of the basal ganglia are GABAergic, and the presence of functional GABA-A and GABA-B receptors in PPTg and LDTg has been pharmacologically and electrophysiologically demonstrated; however, their postsynaptic localization has not been determined yet. PPTg and LDTg comprise intermingled cholinergic, GABAergic and glutamatergic cells, although previous anatomical studies suggest that the targets of pallidotegmental and nigrotegmental projections are largely non-cholinergic cells. As a first step to understand the neural circuitry within these two nuclei we investigated the localization of GABA-A and GABA-B receptors in PPTg and LDTg. Thus, coronal brainstem sections were first processed for NADPH-diaphorase (NADPH-d) staining to establish PPTg and LDTg boundaries, followed by immunoreactivity against either GABA-A receptor alpha 1 subunit (GABAA-alpha1-ir) or GABA-B receptor R2 subunit (GABAB-R2-ir). GABAA-alpha1-ir was light in both nuclei, whereas GABAB-R2-ir was moderately dense in both the neuropil and cell bodies. The potential localization of GABAB-R2-ir in cholinergic (NADPH-d positive) cells observed at the light microscope was confirmed at the confocal microscope using dual immunofluorescence. The quantitative analysis of dually labeled neurons revealed that around 80% of PPTg and LDTg cholinergic cells were also GABAB-R2-immunoreactive (GABAB-R2-ir). Triple labeling using the neuronal marker NeuN showed that 90-95% of GABAB-R2-ir profiles were also NeuN-immunoreactive; thus, in the final series NeuN-ir was not included and all GABAB-R2-ir elements were quantified as neurons. The quantification revealed that GABAB-R2-ir neurons were approximately 2:1 and 4:1 in relation to cholinergic neurons in PPTg and LDTg, respectively. These data provide anatomical evidence of the presence of postsynaptic GABAB receptors in both cholinergic and non-cholinergic neurons in PPTg and LDTg, supporting a potential direct innervation of the two subpopulations by both pallidotegmental and nigrotegmental fibers.

Disclosures: **E. Luquin:** None. **B. Paternain:** None. **E. Mengual:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.06/W31

Topic: D.15. Basal Ganglia

Support: NIH-NINDS grant NS041280

NIH-NINDS grant NS047085

Title: Direct dopaminergic regulation of autonomous and cortically patterned activity in the subthalamic nucleus

Authors: *A. LAHIRI, H.-Y. CHU, M. D. BEVAN;
Northwestern Univ., Chicago, IL

Abstract: The subthalamic nucleus (STN) is a key gatekeeper of information flow through the cortico-basal ganglia-thalamo-cortical circuit. In addition to facilitating cortically driven basal ganglia output via the indirect and hyperdirect pathways, STN neurons exhibit a high degree of autonomous activity and receive direct neuromodulatory input from substantia nigra dopamine neurons that degenerate in Parkinson's disease (PD). We have used a combination of dopamine receptor-selective drugs and optogenetic stimulation of cortico-subthalamic (M1-STN) afferents in mouse brain slices to investigate the effects of dopaminergic neuromodulation on autonomous and synaptically patterned activity in the STN. Exogenous dopamine (0.1 - 50 μM) elicited a dose-dependent increase in the frequency of STN neuron autonomous firing. This effect was reproduced by co- but not individual application of D1-like (SKF 81297) and D2-like (quinpirole) dopamine receptor agonists, consistent with postsynaptic expression of D5 and D2 receptors, respectively. We are currently working to validate these findings through optogenetic stimulation of ChR2(H134)-expressing dopaminergic axon terminals in the STN, and by blocking the dopamine-dependent increase in autonomous activity using D1/5 (LE 300) and D2/3 (sulpiride) antagonists. Motor cortical afferents in STN express D4 receptors and are also subject to neuromodulation by dopamine. Exogenous dopamine reduced the amplitude of EPSC1 upon optogenetic paired-pulse stimulation of M1-STN axon terminals. Application of a D4 agonist (PD 168077) mimicked this effect and also produced an increase in the paired-pulse ratio, suggesting that dopamine can activate presynaptic D4 receptors to reduce the initial probability of M1-STN glutamate release. Experiments are in progress to block this presynaptic effect with a D4 antagonist (L-745,870). With the loss of dopamine in PD, M1 and STN exhibit abnormal, synchronous beta band activity. To test the impact of dopaminergic neuromodulation on cortical patterning of STN activity, M1-STN axon terminals were optogenetically stimulated at 20 Hz for 1 second in the presence and absence of 0.5 μM dopamine. Dopamine significantly reduced cortical excitation of STN neurons (control = 10.6 spikes; dopamine = 4.6 spikes; $n = 9$; $p < 0.05$) while elevating STN autonomous firing (median frequency change = 8.5 Hz).

Together, these findings support a direct role for dopamine in regulating autonomous activity, glutamatergic transmission and synaptic integration in the STN. Loss of direct dopaminergic neuromodulation may contribute to excessive M1-STN patterning in PD.

Disclosures: **A. Lahiri:** None. **H. Chu:** None. **M.D. Bevan:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.07/W32

Topic: D.15. Basal Ganglia

Support: Howard Hughes Medical Institute

Title: Specialized populations of substantia nigra neurons mediate basal ganglia output signaling to brainstem and thalamus

Authors: ***L. E. MCELVAIN**, R. M. COSTA;
Champalimaud Ctr., Lisbon, Portugal

Abstract: The basal ganglia are a network of subcortical nuclei involved in the learning and performance of diverse behavioral actions. Despite their broad importance for behavior, how the basal ganglia ultimately influence downstream circuitry to mediate specific motor, cognitive, and emotional functions remains poorly understood. Here, we investigate the largest output nucleus, the substantia nigra pars reticulata (SNr), to delineate the organization of basal ganglia efferent signaling. Using a combination of anatomical tracing methods and electrophysiological recordings, we 1) define the complete set of brainstem and thalamic regions targeted by SNr, 2) map the extensive collateralization patterns of SNr projections, and 3) characterize the intrinsic electrophysiological properties and topographical organization of independent SNr projection populations. Anterograde tracing from GABAergic and parvalbumin-positive SNr neurons in mice revealed major projections to the: superior colliculus; inferior colliculus; midbrain, pontine, and medullary reticular formations; PPN; dorsal raphe; and thalamic nuclei VM, VA, MD, and Pf. To determine whether specific SNr cell types project to each target, retrograde tracers were injected into downstream structures, and following a 3-4 day survival, whole-cell patch-clamp recordings were targeted *in vitro* to labeled SNr neurons. All SNr neurons shared several features: spontaneous firing, sustained fast-firing (>50 Hz) capabilities, and linear firing rate responses to depolarizing currents. However, neurons projecting to brainstem targets differed significantly from each other and exhibited specialized intrinsic electrophysiological characteristics, including differential sensitivity to input currents, capacity to sustain firing, passive properties, and hyperpolarization-gated currents. Brainstem-projecting SNr neurons were topographically organized and, in some cases, morphologically distinct. In contrast, thalamus-

projecting SNr neurons exhibited heterogeneous electrophysiological and morphological properties and were distributed throughout the nucleus. Mapping of axonal collateralizations from specific SNr neurons revealed that projections to thalamus heavily comprised collaterals from specialized brainstem-projecting populations. These experiments define unique populations of SNr neurons, demonstrate specific and extensive collateralization of projections to thalamus and brainstem, and establish a framework for future investigations linking each SNr output channel to its behavioral functions.

Disclosures: L.E. McElvain: None. **R.M. Costa:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.08/W33

Topic: D.15. Basal Ganglia

Support: Université de Montréal (recrutement)

GRSNC (bourse de formation)

Title: Characteristics of neurones in the globus pallidus of the cat during visually-guided locomotion

Authors: *Y. MULLIE, I. ARTO, J. LEONARD, T. DREW;
GRSNC Groupe de recherche sur le systeme nerveux central, Dépt De Neurosciences, Univ. De Montréal, Montreal, QC, Canada

Abstract: One of the correlates of Parkinson's disease is a modified locomotor gait characterized by small, shuffling steps and a difficulty in turning. Later stages of the disease are frequently accompanied by freezing of gait. However, despite these effects on locomotion we have little information on the characteristics of neurones in different parts of the basal ganglia circuit during locomotion. In the current study we examined the activity patterns of neurons in the globus pallidus (equivalent to the external segment of the globus pallidus, GPe, of primates) of 2 cats during unobstructed locomotion and during visually guided locomotion, when cats stepped over obstacles attached to a moving belt. Most neurons showed relatively high discharge rates at rest (cat sitting or standing, average = 31Hz, range 10-72Hz) and during unobstructed locomotion. Only a small percentage of these cells (~20%) showed discharge activity that was modulated at the frequency of the step cycle during unobstructed locomotion. However, when the cats stepped over the obstacle, a larger percentage of cells (~50%) showed changes in discharge activity that were phase-locked to the modified cycle. In most cases, this discharge was related to the modified step in the contralateral forelimb but the discharge activity in a few cells was changed during the step over the obstacle by the contralateral hindlimb. Cells were related to

the contralateral forelimb both when this limb was the first to step over the obstacle (lead condition) and when it was the second (trail condition). In this respect the changes are similar to those observed in the motor cortex (Drew 1993). However, in all task-related cells, changes in discharge activity lasted throughout the swing period rather than being fractionated as in the motor cortex in the same task. The results suggest a function for the GPe in modulating the general features of the locomotor motor program rather than in regulating more specific details, such as intralimb coordination. Y.MULLIE and I.ARTO contributed equally to this work

Disclosures: **Y. Mullie:** None. **I. Arto:** None. **J. Leonard:** None. **T. Drew:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.09/W34

Topic: D.15. Basal Ganglia

Title: superior colliculus activation has opposing influence on parafascicular and medial posterior thalamic nuclei

Authors: *G. D. WATSON, K. D. ALLOWAY;
Ctr. for Neural Engin., Penn State Univ., University Park, PA

Abstract: The superior colliculus (SC) is known to be a part of a brainstem activating system that reorients attention to salient stimuli. Anatomically, SC sends dense projections to both the parafascicular (Pf) nucleus of the thalamus and to the zona incerta (ZI). The SC projections to ZI overlap with GABAergic neurons that project to the medial posterior (POm) nucleus of the thalamus. We have shown that microstimulating SC inhibits spontaneous activity in POm, putatively through ZI. Previous research has also shown that microstimulating SC activates neurons in the centromedian-parafascicular complex. However, the effect SC stimulation specifically has on Pf in rats has not been well characterized. Therefore we stimulated SC while simultaneously recording from whisker-sensitive regions of Pf and POm. We found that activating SC enhances activity in Pf, while activity in POm is attenuated. These findings support the hypothesis that during a salient stimulus, SC orchestrates Pf and POm in an opposing fashion to influence striatal activity.

Disclosures: **G.D. Watson:** None. **K.D. Alloway:** None.

Poster

340. Basal Ganglia output

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 340.10/W35

Topic: D.15. Basal Ganglia

Support: NIH P01 NS044393

CNRN P30 NS076405

Title: Basal ganglia output is not the determinant of movement-related activity in the pallidal-recipient thalamus

Authors: *A. ZIMNIK[1], R. S. TURNER[2];
[1]Neurobio., [2]Neurobio. and Systems Neurosci. Inst., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Most theories of basal ganglia-thalamo-cortical (BG-TC) function assume that this circuit operates by a "gating" mechanism in which movement-related activations of the BG recipient thalamus (the anterior ventrolateral nucleus, VLa) are produced primarily by disinhibitory decreases in firing in the globus pallidus internus (GPi, primary output nucleus of the BG). To test that theory, we recorded single unit activity and local field potentials (LFPs) from the GPi-VLa subcircuit interconnected with primary motor cortex (M1) as determined by the presence of orthodromic and antidromic responses to electrical stimulation in the proximal arm region of M1. We recorded simultaneously from connected regions in GPi and VLa (n=149 and 150 neurons, respectively) in a macaque monkey while the animal performed a choice reaction time reaching task. A comparison of the latencies of movement related changes in firing rate revealed that as a population, changes in VLa firing began earlier than changes in GPi firing (median 52ms lag, $p<0.05$). This temporal relationship was consistent for both sensory-cued and self-initiated movements. Additionally, large proportions of cells in both areas demonstrated peri-movement increases in firing (75% and 86%, respectively) rather than the inverse incidence predicted by the gating hypothesis. Both results are inconsistent with the idea that task-related activity in VLa is a product of GPi-mediated gating. Despite recording from a large number of GPi-VLa pairs (226 to date), only 4% of pairs showed significant spike-to-spike cross-correlations and all of those were of a form inconsistent with a direct driving of VLa activity by decreases in GPi activity. In agreement with our analysis of single-unit data, coherence between LFPs from GPi and VLa was often modulated around the time of movement onset, but the LFPs from VLa phase-led during those changes in coherence. Together, these results suggest that the relationship between GPi and VLa activity is more complicated than the gating model predicts. Our results are consistent with the idea proposed by others that cortico-thalamic inputs are the primary source of VLa task-related modulations in activity. During performance of a well-learned reaching task, the influence of GPi inputs on VLa activity is at most modulatory.

Disclosures: A. Zimnik: None. **R.S. Turner:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.01/W36

Topic: D.16. Posture and Gait

Support: NIH Grant NS090751

NIH Grant NS092241

NIH Grant HD048741

Title: Learning or not? Mechanisms underlying error correction in locomotion

Authors: *R. T. ROEMMICH[1,2], A. W. LONG[1,3], A. J. BASTIAN[1,2];
[1]Motion Analysis Lab., Kennedy Krieger Inst., Baltimore, MD; [2]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Multiple mechanisms are engaged during motor learning, yet it is not understood if and how they interact to improve performance and store new patterns. During walking, people adapt their locomotor pattern on a split-belt treadmill using intrinsic errors signaled by proprioceptive feedback. Yet, extrinsic visual feedback can accelerate error correction during learning. Here we studied how these two error correction processes interact. We collected kinematic data from young adults (n=28) as they walked on a split-belt treadmill. All participants adapted to a split-belt perturbation during which one belt moved twice as fast as the other (Adaptation, belt speeds 1.4 and 0.7 m/s, 10 min) and then underwent a washout period with the belts moving at tied speeds (Deadaptation, belt speeds 0.7 m/s, 10 min). In Experiment 1, the Feedback (FB) group was provided visual feedback of their step lengths at each heel-strike throughout Adaptation. The No Feedback (No FB) group watched a TV show during Adaptation. In Experiment 2, the Short Feedback group was provided similar feedback of their individual step lengths during the first 30 seconds of Adaptation, after which the participants watched TV for the remainder of Adaptation. All groups watched TV during Deadaptation. Participants used the feedback to match their step lengths bilaterally when the feedback was on and walked comfortably when the feedback was off. In Experiment 1, we observed that error correction occurred faster in the FB group than in the No FB group. However, both groups showed similar aftereffects during Deadaptation. We then asked whether faster error correction in the FB group driven by accelerated adaptation or by a different mechanism. In Experiment 2, we found that the removal of visual feedback during Adaptation caused a rapid drop in the amount of error corrected such that performance was similar to the No FB group from Experiment 1. These findings show that visual feedback drives a process characterized by rapid error correction and immediate forgetting whereas a slower, longer-lasting adaptive process builds similarly with or without extrinsic feedback. In sum, we found that intrinsic and extrinsic error feedback drive fundamentally different processes during split-belt treadmill walking. Intrinsic feedback facilitates adaptation, which occurred similarly across all groups. Conversely, extrinsic feedback drives an error correction process that is not adaptation; rather, this is a transient process that

responds to residual error from adaptation but shows no retention in the absence of extrinsic feedback. Supported by NIH grants NS090751 to RTR, NS092241 to AWL, and HD048741 to AJB.

Disclosures: R.T. Roemmich: None. **A.W. Long:** None. **A.J. Bastian:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.02/W37

Topic: D.16. Posture and Gait

Support: NIH Grant HD048741

Title: Variations in sensorimotor recalibration suggest multiple components of locomotor learning

Authors: *M. STATTON[1,4], A. VAZQUEZ[2,4], A. J. BASTIAN[3,4];
[2]Dept. of Biomed. Engin., [3]Dept. of Neurosci., [1]Johns Hopkins Univ., Baltimore, MD; [4]Motion Analysis Lab., Kennedy Krieger Inst., Baltimore, MD

Abstract: Recent work has shown that in addition to recalibrating movement, motor learning can lead to temporary changes in perception of limb position and movement. For example, perceived hand position and movement change after reaching adaptation, and perceived leg speed changes after split-belt walking adaptation. Here we discovered that specific perturbation conditions produce different perceptual aftereffects despite similar motor aftereffects following split-belt treadmill adaptation. Young, healthy adults walked on a split-belt treadmill with the “fast” and “slow” belt speeds set at 1.5 m/s and 0.5 m/s respectively. Subjects were randomly divided into three groups that experienced this perturbation either abruptly, gradually (where the belts both started at 0.5 m/s and the “fast” leg was gradually sped up to 1.5 m/s), or abruptly with an extended adaptation time (allowing for more time spent in the adapted state). Leg speed perception was assessed via a novel leg speed matching task, where subjects were asked to manually adjust the right belt speed while walking to match that of the left belt speed (which was held at a constant speed). Consistent with prior work, split-belt adaptation led to changes in leg speed perception such that the “fast” leg during adaptation feels slower afterward, and vice versa. We also found that different perturbation conditions led to similar amounts of motor adaptation, but gradual adaptation produced significantly larger changes in leg speed perception than abrupt adaptation. Additionally, extended abrupt adaptation tended to produce larger changes in leg speed perception compared to shorter abrupt adaptation. These results suggest that distinct mechanisms are involved in locomotor learning, and we hypothesize that one component alters the perception of leg speed similar to what might be expected from a forward model

computation. Importantly, this component responds to different perturbation schedules and is largest when small errors are experienced. Our results also suggest that at least one other learning mechanism is engaged to fully account for the motor learning. Supported by NIH HD048741.

Disclosures: **M. Statton:** None. **A. Vazquez:** None. **A.J. Bastian:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.03/W38

Topic: D.16. Posture and Gait

Support: NSERC (Discovery grant #312041-2008)

CFI (Leaders Opportunity Fund)

Title: Dynamic postural control and associated attentional demands in contemporary dancers

Authors: G. SIROIS-LECLERC[1], A. REMAUD[3], *M. BILODEAU[1,3,2];
[1]Sch. of Rehabil. Sci., [2]Sch. of Human Kinetics, Univ. of Ottawa, Ottawa, ON, Canada; [3]Bruyère Res. Inst., Ottawa, ON, Canada

Abstract: Purpose: It has been suggested that attentional demands associated with postural control could be decreased if an individual becomes more proficient at performing a given postural task, such as in elite athletes. The aim of this study was to compare dynamic postural control and associated attentional demands between formally-trained contemporary dancers and controls. Methods: Twenty dancers and 16 non-dancers participated in the study. Dynamic postural control was assessed by having participants track a moving target on a screen (target speed = 1.9 cm/s) with the representation of their center of pressure (CoP) by shifting their weight, either side-to-side (ML) or front-to-back (AP), while standing on a force platform. In order to assess attentional demands associated with this postural task, AP and ML postural conditions were performed 1) with no concurrent task or while simultaneously performing 2) simple (SRT) or 3) choice (CRT) reaction time tasks (dual-task paradigm). The reaction time tasks consisted of responding verbally as fast as possible following the presentation of specific auditory tones (SRT: single tone; CRT: high- and low-pitch tones). Postural control was quantified by calculating 1) the average distance (in cm) between the CoP and the target (proximity) and 2) the average CoP velocity (in cm/s) in both the AP and ML directions for each trial. Mixed analysis of variance models were used to determine the effects of mainly: group (dancers/non-dancers), task (single/dual) and direction (AP/ML), as well as their potential interactions. Results: No differences or interactions were observed between dancers and non-dancers with regards to reaction time. For postural control variables, significant group interactions were found. In particular, the proximity value was smaller (closer) for the AP than

the ML direction for dancers, but only for the dual-task trials, whereas such AP/ML difference was present for all conditions in controls. Also, the average CoP velocity was higher in the ML compared with the AP direction in controls, but not in dancers. Discussion: The fact that dancers presented with lesser differences between AP and ML directions compared with controls, particularly for the single task condition, suggests that their training leads to improved dynamic balance control and potentially lesser associated attentional demands.

Disclosures: G. Sirois-Leclerc: None. **A. Remaud:** None. **M. Bilodeau:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.04/W39

Topic: D.16. Posture and Gait

Support: Canada Foundation for Innovation - Leaders Opportunity Fund

Title: Effects of auditory cues on sway during postural tasks of varying difficulty in young adults

Authors: *A. REMAUD[1], C. ALLARY[2], C. DESGAGNÉ[2], D. MURPHY[2], F. THIFFEAULT-GAGNÉ[2], V. VAILLANCOURT[2], C. GIGUÈRE[2], M. BILODEAU[1,2];
[1]Aging and Movement Res. Lab., Bruyere Res. Inst., Ottawa, ON, Canada; [2]Sch. of Rehabil. Sci., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Background. Postural control is usually described as a multisensory process requiring the integration of somatosensory, vestibular and visual inputs. However, some studies have pointed out that the auditory system might also contribute to the control of balance, particularly when vision is removed. While some authors reported a decrease in postural sway when providing participants with auditory spatial cues, others have suggested that such cues could disturb the control of posture. The different postural conditions used in these studies may explain in part these discrepancies. The aim of this study was to examine how auditory spatial cues can influence postural sway during quiet standing tasks of various levels of difficulty. Methods. Twenty participants (11 women; 9 men) were first screened by audiometric testing to ensure they had normal hearing between 250-8000 Hz. They were then asked to stand as still as possible on a force platform during 30-s trials, in a soundproof room. Twelve postural conditions, presented randomly and performed three times, involved combinations of three bases of support (feet together, feet together on a foam surface, single-leg), two visual conditions (eyes opened and eyes closed), and the presence or absence of auditory spatial cues. The latter consisted of two independent sources of white noise delivered at 70 dB by two speakers located on the left and right sides, approximately 0.5 m away from the participant, at ear level. Postural control was

quantified by calculating: 1) the 95% confidence ellipse area (in cm²) of the center of pressure (CoP) displacements and 2) the average CoP velocity (in cm/s) in anterior-posterior (AP) and medial-lateral (ML) directions. Results. The values of the ellipse area did not differ between the silent and sound conditions, regardless of the base of support and visual condition ($p>0.05$). In contrast, higher CoP velocities in both AP and ML directions were observed when auditory spatial cues were added than when the trial was performed in silence, but only in the single-leg stance with eyes closed (AP: 4.89 ± 1.47 cm/s vs. 4.40 ± 0.91 cm/s, ML: 5.52 ± 1.36 cm/s vs. 5.03 ± 0.97 cm/s, respectively; $p<0.001$). Conclusion. This study showed that providing auditory cues (two independent sources of white noise) during postural tasks of easy-to-moderate difficulty does not improve nor disturb postural stability. However, the addition of auditory cues was shown to disturb postural stability during the more challenging postural task.

Disclosures: **A. Remaud:** None. **C. Allary:** None. **C. Desgagné:** None. **D. Murphy:** None. **F. Thiffeault-Gagné:** None. **V. Vaillancourt:** None. **C. Giguère:** None. **M. Bilodeau:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.05/W40

Topic: D.16. Posture and Gait

Support: Saudi Arabia Ministry of Higher Education

Saudi Arabian Cultural Mission in United States

Title: The effect of cognitive manipulation and gender on the Timed Up and Go (TUG) test

Authors: R. ALMAJID, *E. A. KESHNER;
Dept. of Physical Therapy, Temple Univ., Philadelphia, PA

Abstract: Tasks that require memory and executive function have been shown to affect functional mobility [1,2]. The purpose of this study was to determine if the TUG test could be modified so that it presented attentional demands similar to real-world situations. Five cognitive tasks were performed while seated and during the TUG test to determine effect size and gender differences in 8 healthy male and 8 healthy female adults (25.8±4.9 yrs). Cognitive demands included verbal, mental, reaction, auditory Stroop, and memory tasks. MANOVA was used to compare cognitive performance across tasks, and repeated measures ANOVA was used to compare gait speed, cadence, number of steps, and time to completion. All subjects took significantly more time to complete the modified TUG ($p<0.001$) than the unmodified TUG except with the reaction time task. Gait speed was slower in the modified TUG tests ($p=0.003$) except during reaction time and auditory Stroop tasks. Subjects took significantly more steps ($p=0.01$) with all but reaction time and mental tasks. Results also suggest a gender difference in

cognitive performance both during sitting and the TUG. Women have faster response rates than men in a memory task during sitting (p=0.005) and men are faster than women in the mental task during the TUG test (p=0.01). Women had a significantly higher cadence than men (p=0.008). Although not significant, it was observed that men took greater time via a slower gait speed and fewer steps during modified TUG conditions. Results suggest that TUG combined with cognitive tasks would present a more functional measure of gait performance than the standard TUG test. Cognitive tasks that produce mental interference were more detrimental to spatiotemporal measures of gait. References 1.Al-Yahya E et al. (2011). Neuroscience & Biobehavioral Reviews, 35(3), 715-728. 2.Coulthard JT et al. (2015). Gait & posture, 41(4), 882-887.

Disclosures: R. Almajid: None. **E.A. Keshner:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.06/W41

Topic: D.16. Posture and Gait

Support: Japan Society for the Promotion of Science KAKENHI Grant 25870131

Emerging Team Grant (the Multidisciplinary SensoriMotor Rehabilitation Research Team) funded by the Canadian Institutes of Health Research (CIHR)

Title: Neural correlates of attentional demands associated with dual-task walking

Authors: *S. SANGANI[1], T. KURAYAMA[2], J. FUNG[1,3];
[1]Feil/Oberfeld/CRIR Res. Ctr., Jewish Rehabil. Hosp., Laval, QC, Canada; [2]Chiba Univ., Chiba, Japan; [3]Sch. of Physical and Occup. Therapy, McGill Univ., Montreal, QC, Canada

Abstract: Walking while simultaneously performing another task requires divided attention, eg. holding a cup without spilling. Stability control during gait alone requires attention, which may be compromised as the cognitive demand increases. The impact of dual-tasking on biomechanical and neural components is yet to be explored. Our primary goal was to investigate gait pattern changes and the neural correlates of complex dual-task walking using functional near-infrared spectroscopy (fNIRS). Healthy young adults (n=11) and a stroke participant walked on a 3m long force-sensing treadmill (CMill, Motek-Forcelink). Cortical activation was acquired with a NIRScout system (NIRx) using a custom-built cap covering the frontal cortex. The protocol included repeated block trials consisting of four alternating blocks of standing (20s) and walking (25s) at a comfortable speed determined prior to the experiment. Five walking trials were performed, each consisting of four randomized conditions including holding a Styrofoam cup that was empty or filled with water, jelly or hot liquid. Participants held the cup in the dominant or non-paretic hand. Primary outcomes included stride length, step width, stride

duration, center of pressure displacements and gait variability (% coefficient of variation in stride duration). The cortical hemodynamic response was quantified by concentration changes of oxygenated hemoglobin (oxyHb) in the frontal cortex. Cortical response maps were determined based on the general linear model using SPM (nirsLAB). Walking with a cup filled with hot liquid was associated with a slight decrease in step width and gait variability in all healthy participants but not the stroke individual. The decrease in step width suggests that all subjects adapted to the back-and-forth slosh frequency of the fluid by adjusting their gait so as to suppress the resonant slosh frequency thereby preventing any spillage. In healthy controls, walking while holding jelly was associated with activation of the supplementary motor area (SMA), whereas holding hot liquid resulted in activation of the premotor cortex (PMC) and dorsolateral prefrontal cortex (DLPFC), which are associated with selective attention. Cortical activation in the stroke participant demonstrated increased activation in the contralesional DLPFC and medial SMA while walking and holding either jelly or water. Absence of significant changes in biomechanical gait parameters suggests that during complex dual-task locomotion, the brain can allocate the required cortical resources to account for increased attentional demands without modifying the inherent locomotor pattern.

Disclosures: **S. Sangani:** None. **T. Kurayama:** None. **J. Fung:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.07/W42

Topic: D.16. Posture and Gait

Title: Effects of partially removed visual feedback on gait symmetry

Authors: ***S.-J. KIM**[1], K. GRAHAM[2];
[1]Mechanical & Bioengineering, [2]Bioengineering, California Baptist Univ., Riverside, CA

Abstract: Patients suffering from neurological disorders often receive treadmill training as part of their rehabilitation. Incorporating elements of visual feedback (VF) have been shown to increase the effectiveness of treadmill training. We have previously shown that the distortion of VF of step length entail unintentional modulations in gait symmetric pattern. In this paradigm, the VF consisted of two vertical bars representing the subject's right and left step lengths displayed on a computer screen and also distorted so that subjects perceived their step length as being asymmetric during treadmill walking. We found that a gradual distortion of VF systematically modulated gait step length away from symmetry. In this study, we explored the effect of a different paradigm of VF by investigating whether the absence of a portion of VF had any impact on gait patterns. In pilot experiment, ten healthy subjects were asked to watch the screen and walk comfortably on the treadmill for two 8-minute trials. The treadmill's speed was

changed during both trials. The first trial increased in speed from 1.3 mph to a peak speed of 2.7 mph midway through the trial, before decreasing in speed to 1.3 mph once more. The second trial began at 2.7 mph and decreased to 1.3 mph midway through the trial before increasing back to 2.7 mph. All the speeds were adjusted by 0.2 mph at 30 seconds intervals (the change in speed profile during the first half of the trial exactly mirrored the change in speed profile during the second half of the trial). After 4 minutes (midway through each subject's trial), only the left vertical bar continued to be displayed. This change in visual perception caused each subjects to modulate his or her gait symmetry. However, subjects displayed two different trends. The more prominent trend (64% of trials) was one in which subjects' right steps became longer than their left steps. The less prominent trend (36% of trials) was that subjects' right steps became shorter than their left steps. We also found that subjects had a greater response at lower walking speeds, which suggests that the observed gait asymmetry may not be simply an aftereffect produced in response to any change in VF since the slower speeds were conducted at different times between the two trials. This result suggests that when the method of VF is altered, such as in displaying step length information for only one leg rather than both legs, subjects respond by spontaneously modulating their gait. If this continues to be shown true, this technique could be used to alter patient's gait patterns by simply removing a portion of VF. The results of this study may contribute to more effective implementation of VF for treadmill rehabilitation.

Disclosures: S. Kim: None. **K. Graham:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.08/W43

Topic: D.16. Posture and Gait

Support: University of Idaho Seed Grant

CLASS Key Fund

Title: Cognitive factors influence postural alignment

Authors: *J. L. BAER, A. Q. JOHNSON, R. G. COHEN;
Univ. of Idaho, Moscow, ID

Abstract: BACKGROUND: Stooped posture with the head forward of the torso is associated with multiple health problems, including headaches and neck pain, reduced upper body strength and mobility, and impaired postural control. Neither physiological nor ergonomic factors are sufficient to explain the prevalence (two of three adults aged 65 or older) of stooped posture, so we are investigating the influence of cognitive factors. Previous research has linked postural control to cognitive factors (especially executive function and attention), but the link between

postural alignment and cognitive factors is almost unexplored. We recently found a correlation between inhibitory control and upright posture, in both healthy older and young subjects. HYPOTHESIS: Cognitive factors play causal role in maintaining upright posture, both (1) in ongoing tasks, and (2) at the onset of goal-directed action. APPROACH: Young, healthy subjects were presented with simple postural/motor tasks while the level of cognitive challenge was manipulated. 3D motion capture data were collected to analyze the influence of cognitive factors on postural alignment. Study 1: Subjects sat and played an easy and hard computer game for 5 minutes with no motivational instruction, then repeated the task with incentive to focus on either their posture or task performance. Study 2: Subjects walked to grasp a target at either a leisurely or hurried pace, with or without instruction to pay attention to their posture. Cognitive tests and questionnaires were also administered. RESULTS: Overall, subjects sat and stood more upright when given incentive to attend to their posture. Study 1: Instructing subjects to prioritize task performance caused stooped posture only during the difficult task. Stooped posture was positively correlated with both state and trait anxiety, and with neck pain. Study 2: When preparing to walk, stooped posture became evident, regardless of whether subjects were hurrying or not. Poor inhibitory control (false alarms in a go-nogo task) was correlated with stooped posture overall and with increased neck compression in the rushed condition. CONCLUSION: Cognitive factors affected postural alignment both when preparing for movement and during extended computer tasks. Anxiety, task difficulty, and inhibitory deficits were all associated with stooped posture, while attention to posture diminished the effects.

Disclosures: J.L. Baer: None. **A.Q. Johnson:** None. **R.G. Cohen:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.09/W44

Topic: D.16. Posture and Gait

Support: This research was subsidized by Tsurunokai in 2013.

Title: Effects of attentional dispersion on event-related brain potentials and postural muscle activities during unilateral arm abduction with neck flexion

Authors: *C. YAGUCHI[1], K. FUJIWARA[2];
[1]Dept. of Physical Therapy, Fac. of Human Sci., Hokkaido Bunkyo Univ., Eniwa, Japan; [2]Fac. of Sports and Hlth., Kanazawa Gakuin Univ., Kanazawa, Japan

Abstract: A flexed neck position leads to non-specific activation of the brain. These effects were investigated only when attention was focused to the task. Attentional allocation has capacity limitation, and when attention is divided to multiple objects, the allocation to an object

decreases. Moreover, attentional function is affected by activation state. Thus, the brain activation by neck flexion would change according to attentional dispersion. We investigated the effects of attentional dispersion on event-related potentials and postural muscle activities during unilateral arm abduction with neck flexion. Subjects were 13 healthy adults. A visual cue signal (S1) was presented for 100 ms around the fixation point. At 1 s after S1 onset, a visual imperative stimulus (6°×6° checkerboard, S2) was presented for 150 ms at 9° to the left or right from the fixation point. Interval between S1s was 3.5 s. S2 comprised target and non-target stimuli presented at the position indicated by S1. If S1 simultaneously indicated two positions, S2 was presented unpredictably at one of the two positions with the equal probability. Subjects covertly focused attention on the position (attentional focusing) or divided attention to two positions (attentional dispersion). In response to the target S2, subjects abducted their right arm at maximum speed. This task was performed with the neck resting and flexion postures. The onset time of postural muscles with respect to middle deltoid onset and the following components of event-related brain potentials were measured. P1-N1, N2 and P3 components were analyzed as indices of the sensory, perceptual and cognitive processing of S2, respectively. The late component of contingent negative variation (CNV) was used to evaluate motor preparation before S2 and anticipatory attention directed to S2. With attentional focusing, CNV potential significantly increased, and N2 and P3 latencies shortened by neck flexion ($p < 0.05$). With attentional dispersion, P1-N1 amplitude significantly increased by neck flexion ($p < 0.05$). The onset time of postural muscles became earlier with both attentional focusing and dispersion ($p < 0.05$), but the earliness was significantly larger with attentional focusing than with attentional dispersion ($p < 0.05$). These results suggest that by neck flexion, the brain areas related to higher cognitive processing, anticipation of S2 and motor preparation were activated with attentional focusing, and that of sensory processing of S2 was activated with attentional dispersion. These changes of the sensory-motor processing by neck flexion would relate to the earliness of onset time of postural muscles.

Disclosures: C. Yaguchi: None. **K. Fujiwara:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.10/W45

Topic: D.16. Posture and Gait

Title: Coherence of center-of-mass and center-of-pressure velocities may reveal influence of increased attentional demand on postural control

Authors: *K. TERRY, J. TO-ALEMANJI, P. JO;
Rehabil. Sci., George Mason Univ., Fairfax, VA

Abstract: This study examined the interaction between postural control and attentional demand as quantified by the dynamics of the body's center of mass (COM) and each foot's center of pressure (COP). After screening 19 healthy participants (6 F/13 M, 24.4 ± 4.7 yrs.) for balance and mobility deficits , each individual stood quietly with eyes open (EO) or closed (EC) on either the floor (FL) or a 2-1/2" thick foam pad (FM). Additionally, attentional demand was increased by having each individual also maintain their standing balance while counting backward by 10 or 7. Each of the 12 condition combinations (2 vision x 2 surface x 3 counting) was repeated 3 times for 36 trials. COM displacement, velocity, acceleration were measured using an integrated measurement unit placed on the lumber spine (APDM, Inc.). COP locations were measured using a pair of pressure-mapping shoe insoles (Pedar, Inc.). COP velocity was calculated using a central difference derivative. To examine COM-COP interaction, COM-COP velocity coherence was calculated and sorted by each condition combination. Overall, group mean coherences for both feet were significantly lower for the EO-FL as compared to the EC-FM condition when no counting was performed. However, for both counting tasks, this difference was no longer present. For the right foot (the dominant foot for all but one participant), counting tasks produced significantly higher EO-FL COM-COP coherences that were also similar to those for the other three vision/support surface combinations. Likewise, there were significant regressions between COM-COP coherence and COM rms velocity ($R2=0.22-0.26$, $p<0.001$) for the EO-FL condition while counting. These findings indicate additional volitional postural control for the EO-FL condition may be required as attentional demand is increased. Conversely, the lack of significant differences for the other three vison/surface combinations indicates that the introduction of increased attentional demand did not significantly affect postural control and that postural control was prioritized over performance of the counting task. Also, despite the much slower counting rate for the count by 7 condition, there were no significant differences in coherence, which suggests that counting difficulty was not a moderator of attentional demand effects on postural control. Caution should be taken in interpreting these results, however, as comparison of each participant's mean coherences and bootstrapped confidence intervals revealed unique relationships between coherence and various standing balance conditions, suggesting that attentional demand effects on postural control are highly individual.

Disclosures: K. Terry: None. **J. To-Alemanji:** None. **P. Jo:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.11/W46

Topic: D.16. Posture and Gait

Title: Dissociation of parietal cortex contributions to obstacle memory in walking cats

Authors: *C. WONG[1], K. G. PEARSON[3], S. G. LOMBER[2];
[1]Grad. Program in Neurosci., [2]Brain and Mind Inst., The Univ. of Western Ontario, London, ON, Canada; [3]Univ. of Alberta, Edmonton, AB, Canada

Abstract: A working memory of environmental obstacles is essential for avoidance in walking mammals. In quadrupeds, vision is unavailable to guide hindleg stepping over an obstacle previously cleared by its forelegs. Instead, visual information about the obstacle and motor information about foreleg clearance are held in memory to modify hindleg movements. Previous studies suggest that this memory system relies on parietal areas associated with sensorimotor integration. To examine the role of parietal cortex in obstacle memory, cortical cooling was used to reversibly deactivate areas 5 or 7 in cats trained to step over an obstacle with their forelegs and pause for a variable delay period before resuming locomotion. While visual input of the obstacle was permitted to guide foreleg clearance in some trials, a tactile variation of the test relied on tactile input to the forelegs to guide hindleg stepping over the remembered obstacle in the absence of visual input. Hindleg step height and trajectory over the obstacle were measured to assess memory. In both visual and tactile variations, bilateral deactivation of area 5 resulted in significantly lower steps and altered trajectories, demonstrating a disregard for the obstacle. In contrast, hindleg stepping was unaffected when area 7 was bilaterally deactivated in both variations. When area 5 cooling was restricted to the delay phase when obstacle memory must be maintained, similar stepping deficits were observed regardless of sensory input type. In the tactile obstacle memory test, restricting area 5 cooling to the memory acquisition phase (approach and foreleg clearance) also produced similar memory deficits, demonstrating the necessity of area 5 to all phases of the tactile test. However, obstacle memory was restored when deactivation was limited to the memory acquisition phase of the visual test. Furthermore, when area 5 was deactivated and reactivated within the memory maintenance phase, stepping was demonstrative of intact obstacle memory. Inputs from other areas may be responsible for restoring obstacle memory if area 5 is reactivated during memory maintenance. Together, these results demonstrate that area 5 is necessary, but not sufficient for visually-guided obstacle memory.

Disclosures: C. Wong: None. **K.G. Pearson:** None. **S.G. Lomber:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.12/W47

Topic: D.16. Posture and Gait

Title: Effects of light-induced modulation of the neuronal activity in the lateral cerebellar nucleus on locomotor-related discharges of motor cortex neurons in the cat

Authors: *V. MARLINSKI, I. N. BELOOZEROVA;
Barrow Neurolog. Institute. Div. of Neurobio., Phoenix, AZ

Abstract: During locomotion, the activity of neurons in the lateral cerebellar nucleus (LCN) is modulated with the step cycle. The LCN gives rise to cerebello-thalamo-cortical pathways, which are hypothesized to convey locomotor-related signals to motor cortex (MC). The goal of this study was to elucidate impact of LCN activity on locomotor-related discharges of motor cortex neurons. A cat was trained to walk on the treadmill, and surgically prepared for chronic experiments. Using extracellular recordings, hindlimb-related areas in the LCN and MC were identified. The LCN area was infected with a 50/50 mixture of rAAV5-CAG-ArchT-tdTomato and rAAV5-CAG-ChR2-GFP (UNC Vector Core): 6 injections of 1 µL spaced 0.5 mm apart. Two months later, 6 fiber-optic cannulae (125 µm, Doric) were inserted into sites of injections. During recording experiments, cannulae were connected to lasers (MGL-FN-561nm-100mW and MBL-III-473nm-150mW, OptoEngine) via an optical cable (ThorLabs). During locomotion, the activity of MC neurons was recorded, while the LCN was transiently illuminated with yellow (1.3 s pulses, 10 mW) or blue light (1.3 s trains of 2 ms 50/s pulses, 10 mW). Illumination intensity was verified with the Optical Power Meter (ThorLabs). Postmortem histological examination revealed that, on each of 40 µm sections through the LCN, ~10 neurons located within 1 mm distance from the injection side expressed tdTomato, and a few neurons and glial cells expressed GFP. All labeled cells were located in the dorsal or dorso-lateral part of the LCN extending 1 mm anterio-posteriory, 1 mm medio-laterally, and 0.5 mm vertically. Effects of light-induced modulation of the activity of the LCN on the firing behavior of 19 MC neurons during locomotion were analyzed. Nine MC neurons (47%) responded to LCN stimulation. Six changed their activity during illumination with blue light and 6 with yellow light. Three neurons of each group responded to both lights. All neurons responding to blue light increased their firing rate. Changes in the activity of neurons responsive to yellow light were diverse. In 4 of these neurons the firing activity decreased, and in 2 of them increased. In all neurons, differences in the locomotor-related activity were expressed as moderate changes in the average firing rate and/or in the amplitude of stride-related modulation of the firing activity. Temporal patterns of the firing during the stride were not affected. The study shows that optogenetic technique can be successfully used for transient modulation of neuronal activity in the feline brain. The results suggest that even small groups of LCN neurons can effectively modulate the locomotor-related activity in the MC.

Disclosures: V. Marlinski: None. **I.N. Beloozerova:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.13/W48

Topic: D.16. Posture and Gait

Support: NIH Grant NS072651

NIH Grant NS054894

Title: Using reward to access the limits of locomotion in a rodent-robot interaction paradigm

Authors: *D. LOGAN, J. VANLOOZEN, R. ESPAÑA, S. F. GISZTER;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Measures of motor behavior such as number of lever presses or motion speed have been used to study tradeoffs of motivation for reward, effort and value in a neuroeconomic framework. Here we introduce a framework which uses reward-based learning to investigate how much an animal is willing to alter locomotion (i.e., central patterns with high spinal involvement) for reward. We investigated if animals consistently learn to increase the global variable of pelvis height during treadmill locomotion to receive electrical stimulation of the medial forebrain bundle (MFB), and the long term effects of such learning. SD rats (n=5) were implanted with a bipolar stimulating electrode aimed at the MFB and a pelvis orthosis. This pelvis orthosis connected to a gimbal attached to a phantom haptic robot so the pelvis height of the animal could be monitored in real time. Animals received reward (25 pulses at 60 Hz with 2 msec width) when their pelvis reached a specified offset height. Animals were given at least three weeks to recover from implant surgery and then completed 3 days (2x 10 min each day) of locomotor training on a treadmill with no reward given. Following this baseline training, animals began the shaping process of learning to walk with a higher pelvis for reward. This consisted of sessions (~2-3 per week) where a 3 minute baseline trial without reward was followed by 2 minute reward trials where offset height was progressively increased from trial to trial until the animal no longer received a single reward during a 2 minute trial. Offset heights were chosen as intervals (1.75, 2, 2.25, etc.) of each specific animal's standard deviation of pelvic height during the initial baseline trial. Significant change was determined from comparisons (dependent samples t-test, $p<.05$) of pelvis height during reward trials compared to the baseline trial of that session. All animals have shown the capability to successfully and progressively raise their pelvis in the majority (79% on average across animals) of these reward sessions. On average across subjects, animals were able to simultaneous raise pelvis significantly and receive reward at a maximum offset of 3.4 standard deviation units from initial baseline levels. Our long-term goal is to standardize this shaping procedure to investigate the interaction, and potentially effort-value trade-offs, among reward and energetic and coordinative changes in the locomotive behavior. Doing so may provide a model system for studying the neuroeconomics of locomotion, a more automatic component of movement, in relation to novel skills, and a novel way to evaluate the usefulness of reward-driven plasticity in locomotive rehabilitation.

Disclosures: D. Logan: None. **J. VanLoozen:** None. **R. España:** None. **S.F. Giszter:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.14/X1

Topic: D.16. Posture and Gait

Support: Edith and Richard Strauss Fellowship in Rehabilitation Sciences

CIHR Operating Grant MOP - 77548

Title: Goal-directed locomotion in post-stroke unilateral spatial neglect

Authors: *T. OGOURTSOVA[1], P. ARCHAMBAULT[2], A. LAMONTAGNE[2];
[1]McGill University, Sch. of Physical and Occupati, Montreal, QC, Canada; [2]Sch. of Physical and Occup. Therapy, McGill Univ., Montreal, QC, Canada

Abstract: Unilateral spatial neglect (USN), a highly prevalent post-stroke impairment, has been shown to affect the recovery of locomotion. Our current understanding of goal-directed locomotion control in post-stroke USN, however, is poor and existing literature lacks consensus on the expression of the deficits. Goal-directed reaching accuracy to remembered, as opposed to actual seen objects, is also more affected by USN. Whether such distinctions exists for goal-directed walking remains to be explored. We examined goal-directed locomotion abilities to different target locations in chronic stroke subjects with USN (USN+) and without (USN-). Sixteen individuals, aged 56.9 ± 8.1 (USN+, n=9), and 58.6 ± 12.8 (USN-, n=7) participated in the study. Subjects were asked to walk to actual targets and remembered target locations, represented by 3D spheres, located at 7m and at -15, 0, or 15° from the midline, while immersed in a richly-textured virtual room viewed though a head mounted display. Fifteen trials per target condition were performed. Endpoint heading errors (difference in orientation between actual and ideal trajectory) were examined at 5m of forward displacement. Heading errors for the USN- group ranged from 0.4 ± 1.9° to -2.4 ± 3.8° for the actual target condition, and from -0.3 ± 4.7° to -1.0 ± 4.5° for the remembered target condition. USN+ participants displayed heading errors that resembled those of the USN- group for the middle (-1.9 ± 4.3°/-1.9 ± 4.8°, actual/remembered) and right target locations (0.9 ± 5.7°/-1.4 ± 4.9°), both for the actual and remembered conditions. For the left target location, however, they showed larger heading errors (-4.8 ± 2.9° / -4.5 ± 4.8°, actual/remembered) than the USN- group. Findings indicate that the presence of post-stroke USN alters goal-directed locomotion abilities, especially when walking towards targets located in the left (neglected) hemispace. Results collected so far, however, do not support the presence of larger deficits in response to remembered target vs. actual targets, as reported previously for goal-directed reaching. Possible reasons for the latter finding include the use of a different spatial frame of reference in reaching and walking, as well as a different expression of USN in near (within arm reach) vs. far space.

Disclosures: T. Ogourtsova: None. **P. Archambault:** None. **A. Lamontagne:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.15/X2

Topic: D.16. Posture and Gait

Support: NSF 1342183

Title: Does awareness of split-belt perturbation reduces the generalization of treadmill-learning to over ground walking?

Authors: D. M. MARISCAL, P. A. ITURRALDE, *G. TORRES-OVIEDO;
Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Previous studies have demonstrated that awareness of external perturbations affecting our movements results in faster motor adaptation to such perturbations (e.g., Malone and Bastian 2010). Here we ask whether awareness of external perturbations has an effect on the generalization of adapted movements to different situations. We reason that if we are aware of the source perturbing our movements we might be able to contextualize motor corrections to that situation and, thus, generalize less the adapted movements to other conditions. To test this hypothesis, we compared the generalization of movements adapted on a split-belt treadmill to natural over ground walking when awareness of the split-belt (external) perturbation was manipulated. Three groups (n=7 each) were tested with distinct awareness conditions: 1) an "awareness" group (24.0±2.6 yr.), 2) a "distracted" group (26.14± 3.0 yr.), and 3) a control group (24.29±2.7yr.). The "awareness" group walked on the split-belt treadmill while subjects looked at a screen displaying the speed at which each belt was moving. The "distracted" group walked on the split-belt treadmill while subjects performed a cognitive task requiring them to count the number of times they heard a stimulus that appeared at random instances. Finally, the control group walked on the split-belt condition without any visual feedback or distractor. All groups walked over ground and on the treadmill with both belts moving at the same speed for a baseline condition. Then, all groups experienced a gradual split-belt perturbation (i.e., gradual change from 1:1 to 2:1 speed ratio) because this type of adaptation induces generalization (Torres-Oviedo and Bastian 2012) and we tested if awareness of the perturbation would reduced it. Adaptation effects (i.e., after-effects) were assessed on the treadmill when the two belts moved at the same speed and over ground following split-belt walking. Kinematic data were recorded to characterize the after-effects of step symmetry, which is an inter-limb gait parameter known to adapt after split-belt walking (Reisman et al. 2005). One-way ANOVA was used to compare after-effects on the treadmill and over ground across groups. We found that all groups had similar after-effects on the treadmill ($p=0.84$) and over ground ($p=0.73$), regardless of the awareness condition they experienced. Our results indicate that the generalization of adapted movements across different contexts is not affected by awareness of the perturbation adapting our movements. Therefore, context-specificity of motor adaptation is not modulated consciously, but is implicitly regulated by the motor system.

Disclosures: **D.M. Mariscal:** None. **P.A. Iturralde:** None. **G. Torres-Oviedo:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.16/X3

Topic: D.16. Posture and Gait

Support: NIH Grant HD045639

AHA 11SDG7270001

NSF DMS-0928587

Eric P. and Evelyn E. Newman Fund

Gloria Blake Fund

GEM Fellowship

Title: Dynamic entrainment of human walking to external mechanical perturbations

Authors: *J. OCHOA[1], D. STERNAD[3,4,5], N. HOGAN[1,2];
[1]Mechanical Engin., [2]Brain and Cognitive Sci., MIT, Cambridge, MA; [3]Biol., [4]Electrical and Computer Engin., [5]Physics, Northeastern Univ., Boston, MA

Abstract: The concept of a central pattern generator as a fundamental rhythmic movement primitive has found support in animal locomotion. Our recent research on human locomotion demonstrated dynamic entrainment to external periodic perturbations during treadmill walking, evidence of an underlying limit-cycle oscillator. To further explore that oscillator, this study tested the effect of periodic perturbations during overground as well as treadmill walking. Healthy subjects walked overground and on a treadmill while receiving periodic perturbations at the ankle joint via a wearable robot that was programmed to exert short square plantarflexion torque pulses. Prior to the experiment, subjects' preferred stride period was measured and the perturbation periods were set to be 50ms faster and slower than preferred. On each of 4 trials, subjects walked 15 strides without perturbation; 50 strides with perturbation; and continued walking for 20 further unperturbed strides. Subjects performed a distractor task to minimize voluntary adaptation. To assess entrainment and phase-locking, subjects' knee angles were measured. Entrainment to both slower and faster perturbations was observed during both overground and treadmill walking, evidenced by a near-constant phase of maximum knee flexion with respect to the perturbation pulse. Entrainment to faster perturbation periods occurred after 15-20 cycles and with little phase variability. Entrainment to slower perturbations only occurred after 35 perturbation cycles and showed greater phase variability. This pattern was also observed

during treadmill walking, although entrainment to faster perturbations was delayed and accompanied by more phase variability. In all trials phase-locking occurred with the perturbation pulse at 'push-off' (~50% of the gait cycle) suggesting that subjects assumed a phase relation such that the perturbation assisted propulsion. After perturbations were discontinued, the entrained walking period persisted for at least 20 strides in both overground and treadmill walking. These observations indicate the presence of a nonlinear limit-cycle attractor underlying human locomotion. They are consistent with a neuro-mechanical periphery that instantiates a dynamic primitive in the form of a semi-autonomous oscillator. That dynamic primitive appears to be capable of generating rhythmic locomotion with minimal intervention from higher centers of the nervous system. This subtle modification of walking period and its persistence suggests a new avenue for gait rehabilitation of patients with neurological impairments.

Disclosures: **J. Ochoa:** None. **D. Sternad:** None. **N. Hogan:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.17/X4

Topic: D.16. Posture and Gait

Support: Artificial Intelligence Research Promotion Foundation

Title: Computer simulation of a brain-musculoskeletal dynamical model for bipedal walking towards personalized rehabilitation

Authors: *D. ICHIMURA[1,2], S. YANO[2], T. YAMAZAKI[1];
[1]Grad. Sch. of Informatics and Engin., The Univ. of Electro-Communications, Chofu/Tokyo, Japan; [2]Rehabil., Tamagawa hospital, Chofu/Tokyo, Japan

Abstract: Rehabilitation is repetition of trial-and-error. For each patient,physical therapists test different rehabilitation methods until they find an effective one for the specific patient. This process could be long and painful. If we can eliminate the trial-and-error process, this will be beneficial for the patients. A potential way is computer simulation of rehabilitation with a personalized computer model for each patient. It is possible to build a brain-musculoskeletal model that takes conditions of a specific patient into account, and test rehabilitation to the model on a computer by simulation. Thus, once estabilished, a personalized brain-musculoskeletal model will provide a means to reduce the cost and pain in rehabilitation, which would realize taylor-made rehabilitation for individual patients. In this study, we built a brain-musculoskeletal dynamical model, which is composed of simulated central pattern generators that generate a gait pattern for multiple joints, a cerebellar model that predicts timing of foot contact on the ground, and a musculoskeletal model of a body. We conducted computer simulation of bipedal walking

using the model. We introduced certain delay in proprioceptive feedback signals, especially from the foot to detect the ground contact. During the initial phase of walking, the cerebellum learned the timing of foot contact on the ground, and after that stable bipedal walking was realized. We observed that when one hemisphere of the cerebellum was removed, the foot at the damaged side was lifted larger than that at the other side. We also observed that increasing the torque at hip joints of both sides was effective for robust gait, which seems consistent with a traditional rehabilitation method. Finally, we observed similar abnormal foot lifting in cerebellar damaged patients. These results suggest that the cerebellum plays an essential role in smooth gait control, and that it is possible to build a brain-musculoskeletal model for an individual patient for taylor-made rehabilitation.

Disclosures: D. Ichimura: None. **S. Yano:** None. **T. Yamazaki:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.18/X5

Topic: D.16. Posture and Gait

Support: Marietta Blau Grant (Ministry of Science Austria)

Title: Keeping in step: Entrainment of theta and low-gamma activity in the brain with gait rhythm

Authors: *J. WAGNER[1], S. MAKEIG[2], C. NEUPER[1], G. MUELLER-PUTZ[1];
[1]Inst. for Knowledge Discovery, Graz Univ. of Technol., Graz Univ. of Technol., Graz, Austria;
[2]Inst. for Neural Computation, Univ. of California San Diego, San Diego, CA

Abstract: Cortical processes occurring during upright human gait are not yet well studied. Source-resolved analysis of high-density electroencephalographic (EEG) data is a promising approach to monitoring brain activities supporting walking. In two recent studies, we investigated spectral patterns of high-density EEG data from able-bodied volunteers in a robot-assisted gait-training experiment. Using independent component analysis (ICA), we were able to show that lower gamma band modulations (24-40 Hz) in premotor cortex are timed to the gait cycle (Wagner et al., 2012; Wagner et al., 2014). It has been previously shown that oscillations in auditory and motor cortices may entrain to external rhythms, and this mechanism has been connected to the encoding of temporal information. Predictive timing is essential when performing rhythmic movements. We will present results from a third study in which we examined the EEG of participants attempting to step in time to an auditory pacing tone sequence. Subjects had to adapt their step length and rate to shifts in tempo of the pacing stimulus (e.g., following unforeseen shifts to a faster or slower tempo). The analysis revealed that during steady

state walking lower gamma (25-40 Hz) and theta (4-8 Hz) modulations entrain to the rhythm of walking in auditory and motor areas. Calculation of the modulation index (Canolty et al., 2006) shows that the gamma modulation is coupled to the phase of the theta rhythm. Unexpected shifts of tempo in the auditory pacing sequence perturbs these neural rhythms. It has been previously proposed that neural oscillations are hierarchically coupled, i.e. low-frequency cortical oscillations can phase-lock to rhythmic external events while high-frequency cortical activity is largest at phases crucial to processing of the attended event (Schroeder & Lakatos, 2009). We propose that entrainment of theta and low-gamma band modulations to gait rhytm could reflect the temporal processing of external (auditory pacing) and internal (step pacing) events. Coupling of these neural rhytms may be instrumental in step timekeeping.

Disclosures: J. Wagner: None. **S. Makeig:** None. **C. Neuper:** None. **G. Mueller-Putz:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.19/X6

Topic: D.16. Posture and Gait

Title: Destabilization of postural stability in young adults with minimal added load

Authors: *N. R. BIRCHFIELD, N. DOUNSKAIA;
Arizona State Univ., Phoenix, AZ

Abstract: Introduction: Humans often carry loads, either in the hands or on the upper trunk as a backpack. The representation of the body in the vertical position as an inverted pendulum and the complexity of neural and muscular control of balance predict that loads can substantially affect postural stability. This is especially true if the load is above the center of mass. It has been confirmed that loads equal to 10% of body weight and greater disturb postural stability in young adults; however, it is unknown if added loads less than 10% of body weight also destabilize postural control. **Purpose:** To investigate whether loads less than 10% of body weight produce a noticeable effect on postural stability in young adults. **Methods:** Participants included 21 young males and females. Four conditions of load were used, unloaded (baseline), and 1%, 3%, and 5% of body weight. In each loaded condition, half of the load was positioned on each shoulder. Postural stability was assessed by testing functional reach (forward and lateral) and by recording postural sway with the use of a HUMAC (CMSi, Stoughton, MA) balance board. The postural sway was recorded in two conditions: with eyes opened and closed. Following baseline (unloaded) measurements, the load and visual feedback (eyes open/closed) conditions were randomized. Functional reach scores were calculated as reach distance. Mean COP velocity was obtained using kinematic measures of COP in the anterior-posterior (COP_Y) and medio-lateral (COP_X) directions. **Results:** There was a significant effect of load on functional reach in the

forward ($p < .001$) and left medio-lateral ($p = .03$) directions. In the forward direction, pairwise comparisons revealed significant differences between baseline and 1% ($p < .001$), baseline and 3% ($p = .007$), and baseline and 5% ($p < .001$), but no significant differences between the loaded conditions. In the lateral direction, there were significant differences between baseline and 1% ($p = .02$) and baseline and 5% ($p = .03$), but no significant differences between the two. There were significant main effects for load ($p = .004$) and vision ($p < .001$) on mean velocity. No significant interactions were found. Within the loaded conditions, pairwise comparisons revealed significant differences between baseline and 1% ($p = .019$) and baseline and 5% ($p = .017$) but there were no differences between the loaded conditions. **Conclusions**: The obtained results suggest that even minimal loads of 1% perturb balance in young healthy adults. This result emphasizes the complexity of neural and muscular control that provides postural stability in humans and demonstrates its high sensitivity to the mechanical properties of the body.

Disclosures: N.R. Birchfield: None. **N. Dounskaia:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.20/X7

Topic: D.16. Posture and Gait

Support: BMBF 01EO1401

Title: The integration of haptic information for balance support: effects of contact duration and hand dominance

Authors: *L. JOHANNSEN, D. KAULMANN;
Technische Univ. München, München, Germany

Abstract: Fingertip contact with an earth-fixed referent reduces body sway. Few studies addressed the time course of sway during contact transitions. Investigating intermittent touch with only short contact durations, Johannsen et al. (Gait and Posture, 2014) demonstrated that durations of more than 2 s cause slowed recovery of reduced sway to baseline levels. This aftereffect indicates that the sway reduction involves postural control strategies not dependent on concurrent tactile feedback. In the present study we aimed to replicate previous findings by testing not only short, intermittent contact durations but also longer durations resulting in postural steady states. In addition, we assessed the effect of hand dominance on steady-state sway and sway during contact transitions. 16 young adult participants stood with eyes closed on a force plate, were instructed to actively initiate light finger contact on an auditory signal and to keep contact in place until a second signal indicated to cease contact. 5 different contact durations were assessed in partly randomized order within a trial: 1.5, 3.5, 5, 10 and 20 s. The 20

s contact phase always occurred first to prime a steady-state reference postural set with light touch contact. 2 blocks of 6 trials each were conducted with either the dominant or non-dominant hand in random order. Body sway was recorded in terms of Centre of Pressure and trunk kinematics in addition to forces and torques at the contact point. We replicated aftereffects of light touch contact on body sway for contact phases lasting longer than 3.5 s. No difference was found between the 10 s and 20 s phases. Short contact durations (< 5 s) led to an overshoot in sway within 1 s after contact removal. In general, post-contact overshoot and return to baseline levels occurred quicker after contact with the non-dominant hand. Our findings show that contact durations longer than 3.5 s are required to instantiate a postural set for the utilization of light touch for sway control. Dominance of the contacting hand has a general influence on the post-contact sway time course with reduced overshoot and longer aftereffect, which might indicate subtle effects of hemispheric lateralization on sway control with light touch.

Disclosures: L. Johannsen: None. **D. Kaulmann:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.21/X8

Topic: D.16. Posture and Gait

Support: NRC Grant NRCTR-IN13004

NRC Grant NRCTR-IN14006

NRC Grant NRCTR-IN15005

Title: Locomotor adaptation by visual feedback distortion among healthy adults and subjects with stroke

Authors: *J.-H. SHIN[1], W.-K. SONG[1], J. CHUNG[1], S. KIM[2], S.-J. KIM[3];
[1]Natl. Rehabil. Ctr., Seoul, Korea, Republic of; [2]Hanyang Univ., Seoul, Korea, Republic of; [3]California Baptist Univ., Riverside, CA

Abstract: Backgrounds: Various interventions have been tried to alter locomotion in human, and it is especially important among subjects with stroke. Recent studies showed visual feedback could change gait pattern. However, the optimal visual feedback has not been ascertained, and the possibility of locomotor adaptation by visual feedback among participants with stroke was not depicted. Methods: Seven healthy adults and five stroke patients participated in this study. Visual feedback was provided as bar graphs on the screen in front of subjects, which represents stride length of each limb. When participant walks on treadmill with comfortable walking speed, the graph of selected limb was gradually distorted, and they were not aware of the distortion.

Four different types of visual distortion were provided; the distortion gradually increased from 0% to 12% by 2%, 4%, and 6%, and decreased to 0% with the same ratio every minutes. The 2% distortion was also changed every 30 seconds. The visual distortion was given to dominant or non-dominant limb in healthy subject and unaffected limb in stroke patient. Locomotor adaptation was determined by change of bilateral stride length ratio. Results: All of the feedback induced change of bilateral stride length ratio according to the distortion. When the distortion was applied to dominant limb, the 2% distortion induced less change of stride length ratio between limbs compared to other visual distortion. With the distortion to nondominant limb, the 2% distortion which changed every minute induced relatively marked change of bilateral stride length ratio. Among participants with stroke, the stride length ratio was changed with 2% and 4% distortion according to the visual feedback, however 6% distortion did not lead to expected change. Conclusion: We found that a visual feedback with gradual distortion of stride length could induce locomotor adaptation. However, the adaptation differed according to the visual feedback. In addition, the difference was also found between healthy control and subjects with stroke.

Disclosures: **J. Shin:** None. **W. Song:** None. **J. Chung:** None. **S. Kim:** None. **S. Kim:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.22/X9

Topic: D.16. Posture and Gait

Support: Grant-in-aid for JSPS fellows 25-5251

Grant-in-aid for young scientists (B) 26370821

Title: Human walking entrained by brain alternating current stimulation

Authors: S. KOGANEMARU[1], Y. MIKAMI[2], M. MATSUHASHI[2], H. FUKUYAMA[2], *T. MIMA[2];
[1]Kyoto univ., Kyoto, Japan; [2]Human Brain Res. Cntr, Kyoto, Japan

Abstract: Walking is one of the most fundamental movements in animals and human. It requires specific neurons coordinately activating to generatefor regularly patterned motor outputs for of walking. Cerebellum is one of brain locomotor regions as well as midbrain, subthalamus. It can evoke walking-like patterned activities of limb muschles in animal studies. In the present study, we investigated whether transcranial alternating current stimulation (tACS) simulating gait rhythm given over the cerebellum posterior head could alter locomotion rhythm in human. Fourteen healthy subjects participated in the study. They were given tACS over the left cerebellar cortexinion with frequency similar to individual gait cycle, given sham stimulation

and given tACS over skin of scalp during 10-minute walking in a randomized order. As a result, tACS given over the cerebellum posterior head showed a significant entrainment of gait rhythm, compared with sham and skin stimulation. It suggested that the effects of entrainment were probably due to rhythmical activation of brain locomotor region in cerebellum by tACS. Our findings suggest that patterned brain stimulation such as tACS might be a promising method to stimulate brain locomotion center. This is possibly a novel approach for recovery of locomotion function.

Disclosures: S. Koganemaru: None. **Y. Mikami:** None. **M. Matsuhashi:** None. **H. Fukuyama:** None. **T. Mima:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.23/X10

Topic: F.01. Human Cognition and Behavior

Title: Different effect of attentional focus conditions on postural sway parameters during quiet standing

Authors: *K. HAGIO[1], H. OBATA[2], S. SASAGAWA[3], M. SHINYA[2], A. YAMAMOTO[4], K. NAKAZAWA[2];
[1]Grad. Sch. and Col. of Arts and Sci., [2]Dept. of Life Science, Grad. Sch. and Col. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan; [3]Dept. of Human Sci., Kanagawa Univ., Kanagawa, Japan; [4]Dept. of Community Hlth. Sciences, Grad. Sch. of Hlth. Sci., Kobe Univ., Kobe, Japan

Abstract: Many studies have investigated the effect of cognitive tasks on postural stability. However, changes in postural sway reported in the previous studies remains controversial. The reasons include that parameters, represented by center-of-pressure (COP) based measures, to quantify the postural sway are different. The standard deviation of the COP (COPsd) and the mean velocity of COP (COPvel) has been suggested to represent overall postural stability and the amount of activity required to maintain stability, respectively. This study aimed to compare the effect of attentional focus conditions on different center-of-pressure based measures of postural steadiness during quiet standing. Fifty-one young adults participated in this study. They were required to stand on a force platform for 30s and asked to (1) maintain quiet standing (QSt), (2) minimize their postural sway (conscious standing, CSt) and perform a silent mental arithmetic task (calculation, Cal). From the measured ground reaction forces, we calculated the standard deviation (COPSD) and the mean velocity (COPvel) of the center-of-pressure in the anterior/posterior (AP) and the medio/lateral (ML) directions, respectively. The results demonstrated that an internal focus condition (i.e., CSt) and a cognitive task condition (i.e., Cal) on postural control had a different effect on the COPSD and the COPvel, respectively. The CSt

condition increased the COPvel in the AP and ML directions compared to the QSt and Cal conditions. On the other hand, the Cal condition reduced the COPSD in the AP and ML directions compared to the QSt and CSt conditions. These results indicate that postural control mechanisms interfering with a secondary task are different depending where subjects direct their attention.

Disclosures: **K. Hagio:** None. **H. Obata:** A. Employment/Salary (full or part-time):; The University of Tokyo. **S. Sasagawa:** A. Employment/Salary (full or part-time):; Kanagawa University. **M. Shinya:** A. Employment/Salary (full or part-time):; The University of Tokyo. **A. Yamamoto:** A. Employment/Salary (full or part-time):; Kobe University. **K. Nakazawa:** A. Employment/Salary (full or part-time):; The University of Tokyo.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.24/X11

Topic: D.16. Posture and Gait

Title: Effect of time pressure on attentional shift and anticipatory postural control during unilateral shoulder abduction reactions in an oddball-like paradigm

Authors: *K. ANAN[1], K. FUJIWARA[2], C. YAGUCHI[3], N. KIYOTA[4];
[1]Sapporo Intl. Univ., Sapporo, Japan; [2]Fac. of Sports and Hlth., Kanazawa Gakuin Univ., Kanazawa, Japan; [3]Dept. of Physical Therapy, Fac. of Human Sci., Hokkaido Bunkyo Univ., Eniwa, Japan; [4]Dept. of Rehabilitation, Fac. of Hlth. Sci., Japan Hlth. Care Col., Eniwa, Japan

Abstract: In a reaction task with high time pressure, the onset time of postural muscle activity with respect to the focal muscle of arm flexion is later than in low time pressure, and the latter part of P3 waveform varies in a transient choice-reaction task using a finger reaction. However, no studies have investigated the effects of time pressure on P3 waveform in a repeated arm movement task, using the simultaneous evaluation of the behavior and cognitive processing (especially from a viewpoint of attentional shift). Therefore, the effect of time pressure on attentional shift and anticipatory postural control was investigated during unilateral shoulder abduction reactions in an oddball-like paradigm. A visual cue signal (S1) was presented for 100 ms around the centrally located fixation point. At 1 s after S1 onset, a visual imperative stimulus (6°×6° checkerboard, S2) was presented for 150 ms at 9° to the left or right from the fixation point. This S1-S2 sequence was repeated for 100 s in each experimental block. The time interval from S2 to the subsequent S1 was 1.0, 1.5, or 2.0 s. S2 comprised target and non-target stimuli presented at the position (the left or the right) indicated by S1. Right shoulder abduction was performed only in response to target stimuli, which were presented with a 30% probability. The P1, N1, N2, and P3 components of event-related potentials were analyzed, and onset times of

postural muscles were quantified with respect to middle deltoid activation. There was no significant effect of S2-S1 interval on the latency or amplitude of P1, N1, or N2. The percentage of subjects with bimodal P3 peaks was significantly smaller and the slope of the P3 waveform in the 100 ms after the first peak was significantly steeper with a 1.0-s S2-S1 interval than with a 1.5- or 2.0-s S2-S1 interval. The onset of postural muscle activity was significantly later in the shorter interval conditions. These results suggest that with a shorter S2-S1 interval (higher time pressure), attention was allocated to hasten the latter part of cognitive processing that may relate to attentional shift from S2 to next S1, which led to insufficient postural preparation associated with arm movement and anticipatory attention directed to S2.

Disclosures: **K. Anan:** None. **K. Fujiwara:** None. **C. Yaguchi:** None. **N. Kiyota:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.25/X12

Topic: D.16. Posture and Gait

Support: ARO Grant 64929EG

EPSRC Grant EP/H04924X/1

BBSRC Grant BB/J021504/1

Title: Neuromechanics and neurogenetics: old questions and new tools targeted at the control of legged locomotion

Authors: *A. SPENCE;
Bioengineering, Temple Univ., Philadelphia, PA

Abstract: One of the grand challenges for modern science is to understand how animals move. Locomotion results from the dynamic interaction of many complex, nonlinear constituents: the nervous system, muscles, the body, and an often-unpredictable external environment. Yet animals can move quickly, stably, and economically through these challenging environments, relying on these interacting neural and mechanical mechanisms. Here we present an overview of three lines of research that seek to understand distinct but critical aspects of legged locomotion control: 1) comparative work in insects, dogs, and a six-legged robot (the XRL; X-Rhex Lite: Haynes et. al. Proc. SPIE 8387, 2012) aimed at understanding how and why locomotor control strategy (here choice of leg stiffness) varies with body size and leg number; 2) a nonlinear, phase-based approach to modeling gait, that seeks to explain how gait regulation reflects ultimate constraints such as quasi-static stability, and finally, 3) recent work towards bringing optogenetics to bear on problems such as these in intact, freely behaving mice. The

aforementioned lines of research will be used to highlight new avenues of investigation that are being opened up by a burgeoning genetic toolkit and closed-loop experimental designs; historically used to good effect by the flight neuroscience community but less so in terrestrial locomotion.

Disclosures: A. Spence: None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.26/X13

Topic: D.16. Posture and Gait

Support: CFI Grant 9632

Leaders Fund Grant 26562

Title: Execution of reactionary control of posture during an ongoing volitional upper and lower limb task

Authors: *A. H. HUNTLEY[1], K. A. INKOL[1], L. VALLIS[1,2];
[1]Human Hlth. and Nutritional Sci., Univ. of Guelph, Guelph, ON, Canada; [2]Schlegel-UW Res. Inst. of Aging, Kitchener, ON, Canada

Abstract: Incorrect weight transfers during ongoing volitional movement is known to be one main cause of falls, especially in older adults. Previous literature in this area of study has been primarily limited to lower limb tasks, such as gait perturbation studies. Understanding how consistent reactionary control is executed during ongoing volitional tasks involving simultaneous upper and lower limb actions may have important fall prevention implications, as this may be a more representative scenario for activities of daily living that may lead to a fall. In this preliminary study, young adults (n=5) transported a 0.5kg cylindrical object between two, countertop height stands by executing a side step. Support surface perturbations (1m/s^2, 9cm displacement) occurred in 50% of trials (40/80), triggered upon initial touchdown of the subjects' lead laterally stepping foot. Trials involving a sidestep and perturbation in the same or congruent direction were considered SLIP trials, while trials involving a sidestep and perturbation in opposing or incongruent directions were considered TRIP trials. Participants transported the object under two instructed speeds, self-paced and as "quickly and efficiently" as possible. Variability of movement execution and peak velocity was examined based on whole-body kinematic data (OptiTrack, NaturalPoint, USA, 100Hz). Peak center of mass (COM) velocity significantly increased during fast-paced trials compared to self-paced trials ($p<0.001$), confirming that younger adults were able to increase the speed of their movement execution when instructed to, regardless of the challenging demands of the task. Peak COM velocity also

significantly increased from the SLIP condition to the TRIP condition ($p<0.001$), while a trend for coefficient of variation values to increase from SLIP to TRIP conditions was observed ($p=0.061$). This may be due to increased task complexity and level of motor control required by the central nervous system to arrest body motion safely and complete the volitional task, thereby avoiding a fall. Further analyses are underway to examine measures of whole-body stability throughout the volitional and reactionary phases of movement to help quantify variability changes in task execution. This will help elucidate how reactionary control strategies are executed during ongoing volitional tasks to prevent an individual from losing balance, and ultimately falling.

Disclosures: **A.H. Huntley:** None. **K.A. Inkol:** None. **L. Vallis:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.27/X14

Topic: D.16. Posture and Gait

Support: BMBF Grant 01EO1401

Title: Head movement predictability explains suppression of vestibular input during locomotion

Authors: *P. R. MACNEILAGE, S. GLASAUER;
Univ. Hosp. of Munich, Munich, Germany

Abstract: There is accumulating evidence that vestibular afferent signals are partially or fully suppressed during active movement in animals. During locomotion the suppressed vestibular signals are supplemented or even replaced by predictive signals based on efference copies of locomotor commands. Such a strategy is thought to improve estimates of head motion through space for compensatory behaviors such as gaze stabilization. But the value of this periodic and presumably deterministic efference copy for estimating head motion should depend critically on the stereotypy or predictability of the resulting head motion. Here we express this intuition quantitatively in the form of a statistically optimal model in which relative weight given to efference copy and sensory signals depends on motor predictability. We empirically quantify this predictability during human locomotion (running, walking, and walking stairs) by measuring head motion with an inertial measurement unit, and calculating the variance explained by the mean stride-cycle. Head motion predictability is greater during running than during walking, such that efference copy signals should be upweighted and vestibular signals downweighted during running compared to walking. This coincides with reports that impairment of patients with vestibular dysfunction and of normal subjects with vestibular perturbations is less during running than walking. Thus, we provide a theoretical and evidence-based explanation for

vestibular reliance which was previously explained by degree of automatization for different behaviors. The probabilistic model is general and applies to any situation in which achieved movement is estimated from both efference copy and a zero-mean sensory signal with signal-dependent noise.

Disclosures: P.R. MacNeilage: None. **S. Glasauer:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.28/X15

Topic: D.16. Posture and Gait

Support: American Heart Association

Title: Neural mechanisms involved in mental imagery of slipping while walking: A preliminary fMRI study

Authors: *T. S. BHATT[1], P. PATEL[2], S. LANGENECKER[3], S. DELDONNO[3], K. SHARMA[3], L. JENKINS[3];
[1]Physical Therapy, Movt Sci., [2]Physical Therapy,, [3]Psychiatry, Univ. Illinois, Chicago, IL

Abstract: Background: Perturbation induced falls (from trips or slips) are responsible for ~60% of outdoor falls among community-living elderly. Compared with conventional balance training, perturbation training induced via support surface translations (i.e. repeated slips) has proven effective for longer-term falls prevention. While behavioral evidence of motor learning (adaptation & retention) induced via perturbation training is established, the neural correlates underlying such behavioral changes can only been speculated. As a first step this preliminary study *aimed to examine the neural mechanisms involved in reactive balance control for recovery from large-scale environmental perturbations such as slips using mental imagery.* **Method:** Seven healthy young participants after being exposed to one walking and one slip trial performed two mental imagery tasks in the MR scanner. During each MR run including 30s blocks for each condition, participants received verbal instructions to 1) imagine either walking on a treadmill (IW) or 2) imagine experiencing a slip while walking on a treadmill (IS) or 3) rest with vision fixated on a white cross on a black screen. T2* weighted images with echo planer imaging to measure BOLD signals and T1-weighted whole head structural images were acquired. After data pre-processing, level I analysis using GLM was performed to compare the hemodynamic response between conditions for each participant in SPM8. The following contrasts were analyzed: IW vs rest & IS vs IW. **Result:** Subjects received a median score of 4.5/5 on the vividness of visual imagery questionnaire. Results for IW vs rest indicated significant activation of the dominant superior frontal gyrus (premotor area), bilateral superior cerebellar peduncles,

bilateral parahippocampal gyrus, the basal ganglia, and thalamus. Comparing IS to IW, in addition to the dominant premotor area, bilateral superior cerebellar peduncles there was significant activation in the bilateral post central gyrus, posterior cingulate and palteral orbital frontal cortices. **Conclusion:** Results are in concurrence with previous findings examining mental imagery of walking. During mental imagery of an unexpected balance loss and fall from a support surface perturbation (i.e slip), the substrates involved in motor planning, coordination and postural stabilization of internally generated movements were significantly more active.There was also significantly greater activation of substrates involved with internal awareness of body movement and episodic memory retrieval. These findings support the involvement of higher cortical and subcortical structures in reactive balance control.

Disclosures: **T.S. Bhatt:** None. **P. Patel:** None. **S. Langenecker:** None. **S. DelDonno:** None. **K. Sharma:** None. **L. Jenkins:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.29/X16

Topic: D.16. Posture and Gait

Support: CIHR (MOP-77548)

REPAR

JRH Foundation

CNPq

Title: Avoidance strategies in response to animate and inanimate obstacles in young healthy individuals walking in a virtual reality environment

Authors: *W. H. DE SOUZA SILVA[1,3], G. ARAVIND[2], S. SANGANI[4], A. LAMONTAGNE[2];
[2]Sch. of Physical and Occup. Therapy, [1]McGill Univ., Montreal, QC, Canada; [3]Integrated Program in Neurosciences, Montreal, QC, Canada; [4]Feil and Oberfeld Res. Ctr., Jewish Rehabil. Hosp., Laval, QC, Canada

Abstract: Many studies have described obstacle avoidance strategies while walking, either in physical or virtual environments. These studies, however, were limited to the avoidance of inanimate objects (e.g. cylinders) or failed to address the influence of the visual and auditory properties of the obstacle in shaping avoidance strategies. This study aims to describe the extent to which three different types of obstacles (cylinder, visual human-like avatar and visual human-like avatar with footsteps sounds) affect the inherent avoidance strategies in young healthy

individuals. Healthy young adults (n=4, 50% male, aged 24.7 ±3.5 years (mean ±1SD)) were tested while walking over ground and viewing a virtual environment (VE) displayed in a helmet mounted display (HMD) unit (nVisor SX60). The VE, controlled in Caren-3 (Motek medical), simulated a large room that included a target located 11m straight ahead. In addition, three identical obstacles were positioned 7m ahead in three locations facing the subject (40° right, 40° left, and straight ahead). As the subjects walked 0.5m, one of the three obstacles approached them by walking/moving towards a theoretical point of collision located 3.5m ahead at the midline. Meanwhile, the two remaining obstacles moved/walked away from the participants. The ability of the subjects to steer toward the target while avoiding the obstacles was characterized using the 3D position and orientation of the head recorded from reflective markers (Vicon) placed on the HMD. Preliminary findings show a trend towards smaller minimal distances in all directions when interacting with human-like avatars (left: 1.25±0.47**;** center: 1.22±0.20; right: 1.31±0.24) as compared to cylinders (left: 1.49±0.26**;** center: 1.29±0.12; right: 1.52±0.17). The addition of footstep sounds to human-like avatars did not modify minimal distance values compared to when no footstep sounds were provided (left: 1.20±0.39**;** center: 1.71±0.14; right: 1.31±0.28). Onset times of avoidance strategies were similar across all conditions. These findings suggest that participants had equivalent movement perception of the obstacles regardless of the condition displayed. They also indicate that smaller clearances in the presence of human-like entities may occur due to an inherent real life perception of the avatars resulting in actions that rely on strategies applied during daily locomotion. Finally, the similarity of results following the addition of footstep sounds to the visual human-like avatar condition suggests that avoidance strategies may primarily rely on visual cues. **Key Words:** Navigation; Vision; Walking.

Disclosures: W.H. De Souza Silva: None. **G. Aravind:** None. **S. Sangani:** None. **A. Lamontagne:** None.

Poster

341. Gait and Posture: Higher Order Control, Multi-Task Integration and Theory

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 341.30/X17

Topic: D.17. Voluntary Movements

Support: NRF grant (no.2014R1A2A2A04003858)

CISS grant (no.20110031867)

Title: Movement-related cortical activities during self-paced and externally-cued gait initiation

Authors: *K. CHA[1], J. CHOI[1], J.-I. SONG[2], J.-H. PARK[2], H.-S. JEON[2], K. KIM[1];
[1]Yonsei Univ., Wonju City, Korea, Republic of; [2]Dept. of Physical Theraphy, Yonsei Univ., Wonju, Korea, Republic of

Abstract: Self-paced and externally-cued movement initiations are controlled by strategies in the central nervous system, but the mechanisms remain unknown. In order to compare control mechanisms between the two different movement initiations, we analyzed the movement-related electroencephalograms (EEGs) during self-paced and externally-cued gait initiations (GIs). The spatiotemporal characteristics of cortical sources were compared between the two GI conditions. 17 volunteers with no record of neuromuscular disorders were enrolled in this study. Subjects were requested to perform an immediate forward gait in response to a visual cue sign, or a self-paced forward gait after the visual cue. During the task performance, 32-channel EEGs and force platform signals were recorded. The EEG waveforms were segmented in -5000~6000 ms interval based on the gait onset determined by the force platform signal for each trial. In addition to the averaged movement-related (MP) waveform analysis, low resolution brain electromagnetic tomography (LORETA) analysis was performed to observe movement-related cortical activities. At -400~0 ms period, Bereitschaftspotential (BP) was observed at central areas for both initiation conditions. The BP amplitude was significantly higher for the self-paced GI than for the externally-cued GI. The LORETA results showed prominent cortical sources at paracentral lobule for both GI conditions. The source current density was significantly higher for the self-paced GI than for the externally-cued GI, especially at medial frontal gyrus. Considering that pre-movement activities in frontal and supplementary motor area are devoted to the preparation and planning of movement, the increased BP amplitude and frontal source current in self-paced GI may reflect the higher cognitive load required for determining the type and timing of movement.

Disclosures: K. Cha: None. **J. Choi:** None. **J. Song:** None. **J. Park:** None. **H. Jeon:** None. **K. Kim:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.01/X18

Topic: D.17. Voluntary Movements

Title: Behavioral effects of transcranial direct current stimulation to the supplementary motor area during motor tasks

Authors: K. E. HUPFELD[1], *C. J. KETCHAM[1], H. D. SCHNIEDER[2];
[1]Exercise Sci., Elon Univ., Elon, NC; [2]Yale Med. Ctr., New Haven, CT

Abstract: Low-cost, portable, and easy-to-use, a technique called transcranial direct current stimulation (tDCS) holds great promise as a novel therapy for treating motor impairments in neurological patients (Madhavan & Shah, 2012). tDCS passes low-intensity current between two electrode sponges_the anode and cathode_placed on the subject's head, which facilitates or inhibits neuronal activity by delivering weak electrical currents to specific brain regions, causing different behavioral outcomes depending on the area stimulated (Schestatsky, Quezada, & Fregni, 2013; Madhavan & Shah, 2012). tDCS has recently been investigated as a therapeutic intervention for treating conditions including aphasia, Parkinson's disease, and stroke_particularly as an augmentative treatment to incorporate into physical, occupational, and speech therapy programs to accelerate motor learning (Schestatsky et al., 2013; Brunoni, Boggio, Bikson, Bolognini, Nitsche, Fregni, & Priori, 2012). This study aimed to investigate the role of tDCS in facilitating motor planning. This study examined the effects of applying anodal tDCS to the supplementary motor area (SMA) on motor planning through administering a battery of motor planning tests, which included a simple reaction time test, balance task (Biodex mCTSIB test), and grooved pegboard task to a population of college students. Participants received either sham stimulation or between 20 and 25 mA•min of current to the SMA and performed each motor test before receiving tDCS at 20 minutes, 40 minutes, and 48 hours. Preliminary results showed college students improved on motor tasks following tDCS application to the SMA. This suggests that the SMA is involved in formulating and executing motor plans and that tDCS may be utilized to strengthen the neural pathways associated with the SMA for motor tasks. These findings have future implications for designing therapeutic interventions for individuals with movement decrements.

Disclosures: K.E. Hupfeld: None. **C.J. Ketcham:** None. **H.D. Schnieder:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.02/X19

Topic: D.17. Voluntary Movements

Support: NWO VICI

Title: Vestibular modulation of hand choice

Authors: *R. S. BAKKER, R. H. A. WEIJER, L. P. J. SELEN, W. P. MEDENDORP; Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Our brain continuously decides which hand to use to perform an action. A critical factor in this competition between the hands is some assessment of the expected effort and expected task success associated with each hand (Schweighofer et al. 2015). These costs and

rewards may depend on the relative position of the hand to the target, biomechanical factors, accuracy demands or handedness (Cisek 2007; Oliveira et al. 2010; Schweighofer et al. 2015). If this competition is solely resolved based on biomechanical costs, hand choices should be symmetrically distributed around the body midline when the body is stationary. On the other hand, if the whole body is under acceleration, hand choices should be biased due to changes in biomechanical costs. Here, we set out to examine hand choices in a unimanual reaching task, both stationary and under whole body acceleration. The inertial forces induced by the whole body motion differ between the arms. This introduces differences in both the biomechanical cost (i.e. effort) and the accuracy (i.e. reward) associated with reaches of the individual arms. Right-handed subjects were seated on a vestibular sled that oscillated sinusoidally over 30 cm at a frequency of 0.71 Hz. Subjects made unimanual reaches to a body fixed target presented on a LED table mounted in front of them. One out of thirteen targets, organized in a semicircular array, was presented in a single trial. Once the target appeared, subjects had to reach as fast and accurate as possible with either hand. Targets were presented both at peak acceleration and peak velocity of the sinusoidal platform motion. Every target was presented 24 times for each condition, from which we calculated the PSE for that phase angle of the sled motion. These choice trials were interspersed with catch trials in which two targets were presented simultaneously and both arms had to move, as well as fixation catch trials in which subjects had to move both hands to the fixation light. The same experiment was also carried out while the subject was stationary, i.e. without acceleration induced inertial forces. Preliminary results of the stationary condition show a bias towards choosing the right hand, as shown by a mean PSE left of the body midline. While in motion, this PSE systematically shifts when the target is presented at peak acceleration, but remains unaffected at peak velocity. Currently, we are interpreting these findings in terms of the effects of whole body acceleration on the computation of the expected effort and expected task success.

Disclosures: **R.S. Bakker:** None. **R.H.A. Weijer:** None. **L.P.J. Selen:** None. **W.P. Medendorp:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.03/X20

Topic: D.17. Voluntary Movements

Support: CAPES Scholarship

FAPESP Grant # 2012/19943-0

Title: Stroke individuals have preserved multi-joint coordination during arm reaching movements in upright position

Authors: *S. M. FREITAS, C. A. LIMA, A. M. S. BALDAN, S. R. ALOUCHE;
Univ. Cidade De Sao Paulo, Sao Paulo, Brazil

Abstract: Individuals with mild post-stroke hemiparesis have several functional limitations in reaching objects during upright standing. It is unknown, however, whether such limitations are due to different patterns of joint coordination used to reach a target during the upright stance. Therefore, the aim of the present study was to investigate the joint coordination patterns used by stroke individuals to reach a target. Fifteen individuals with hemiparesis (eight on the right and seven on the left side) and eight healthy individuals stood in upright position and performed reaching movements towards a target shown in the center of a monitor. The monitor was placed at a distance of 115% of the upper limb's length. Stroke individuals used the arm ipsilateral to the brain lesion to perform the tasks. The patterns of joint coordination were evaluated in certain (target final position was known) and uncertain (target could move to an upper or lower position after the participant started to move) conditions. Each participant performed 5 trials for each target position and condition. Only the trials for the movements performed towards the center target were analyzed. Joint kinematics of the focal movement (shoulder, elbow and wrist) and postural adjustments (hip, knee and ankle) in the sagittal plane were recorded. Principal components (PC) analysis was carried out on the variance of joint angles to investigate the coordination patterns used 150 ms before (postural adjustments phase) and during the movement by stroke and healthy individuals. The uncertainty in target position increased the amplitude of focal joint excursions during both the postural adjustment and movement phases. Only the individuals with right hemiparesis reduced the amplitude of the ankle joint in the uncertain compared to certain condition. The first PC accounted for more than 90% of the total joint variance for all groups suggesting that two joint coordination patterns were implemented simultaneously, one related to the focal movement and other to the postural adjustments. The accounted variance of the first PC was greater during the uncertain compared to certain condition only for the postural adjustment phase. Before and during the movement, the joints of focal movement contributed most for the first PC compared to the other joints. Overall, the results suggest that uncertainty in target position during reaching in an upright position influences the amplitude of joint excursions for both focal and postural movements. However, it does not affect the multi-joint coordination patterns. Stroke individuals also have preserved multi-joint coordination during arm reaching movements in upright position.

Disclosures: **S.M. Freitas:** None. **C.A. Lima:** None. **A.M.S. Baldan:** None. **S.R. Alouche:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.04/X21

Topic: D.17. Voluntary Movements

Support: FISM - Cod. 2013/R/5.

Ministry of Foreign Affairs, Unit for S/T cooperation.

Marie Curie Integration Grant FP7-PEOPLE-2012-CIG-334201 (REMAKE)

MAE - PRG00174

Title: Muscle synergies and kinematic measures in subjects with multiple sclerosis during reaching and manipulation tasks

Authors: *L. PELLEGRINO[1], M. MARGIT[2], C. SOLARO[3], M. COSCIA[4], M. CASADIO[2];
[1]Dept. Informatics, Bioengineering, Robotics and Systems Engin., Univ. of Genoa, Ge, Italy;
[2]Dept. Informatics, Bioengineering, Robotics and Systems Engin., Univ. of Genoa, Genoa, Italy;
[3]ASL3 Genoa, Genoa, Italy; [4]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Abstract: According to several studies, muscles participate to the generation of movements in well-defined functional groups - called "synergies" - activated by descending cortical commands. A recent study has suggested that muscle synergies can be considered as physiological markers of motor cortical damage. The potential of this approach has not yet been exploited for understanding and describing the motor impairments caused by Multiple Sclerosis (MS). This study aimed at evaluating the muscle synergies and kinematic parameters of both upper limbs in MS subjects while they control their upper arm motion and/or forces under different environment conditions:(i) free space; (ii) rigid constrain; (iii) in presence of a spring that opposes the subjects' movement; (iv) in presence of a force field that attracts the hand of the subject toward the target. Subjects were seated on a chair grasping the handle of a planar manipulandum. The robot measured the end-effector position and provided the interactions forces. The subjects reached targets presented in random order in 8 directions equally spaced from a central target (distance: 14 cm or 10 N). The activities of the following 15 muscles of both arms were recorded: triceps brachii long and lateral, biceps brachii short and long head, brachialis, brachioradialis, pronator teres, infraspinatus, latissimus dorsi, upper trapezius, rhomboid major, pectoralis major, anterior deltoid, medial deltoid and posterior deltoid. Seven healthy subjects without neurological or muscular disorders (2 M- 5 F; 53±10 years) and seven subjects with clinically definite MS (2 M - 5 F; 54±8 years) according to McDonald criteria participated in the study. The inclusion criteria for SM were: (i) stable phase of the disease (no relapses in the last three months), (ii) relapsing - remitting (RR) form, (iii) Expanded Disability Status Scale (EDSS) ≤ 7, (iv) Ashworth scale< 2 and (v) 9-HPT >30. As expected control subjects generated straighter and smoother trajectories compared to the MS subjects. For both groups the most difficult task was the control of the isometric force against a rigid constraint. Muscle synergies analysis confirmed that the activity of the recorded 15 muscles could result from the combination of a limited number of basic modules, for both subjects' populations and for both arms. However, in MS subjects muscle synergies had a different structure in the two arms.

Disclosures: **L. Pellegrino:** None. **M. Margit:** None. **C. Solaro:** None. **M. Coscia:** None. **M. Casadio:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.05/X22

Topic: D.17. Voluntary Movements

Title: Neurocomputational cost minimization accounts for the leading joint hypothesis

Authors: *N. DOUNSKAIA, Y. SHIMANSKY, W. WANG;
Arizona State Univ., Phoenix, AZ

Abstract: Although applications of the optimal control framework to human movements have been fruitful, the optimality criteria are still under debate. Minimization of muscle energy is considered most frequently. Surprisingly, the cost of information processing necessary for movement control [neurocomputational cost (NC)] has rarely been discussed. We propose that the tendency to reduce NC accounts for the empirically-derived leading joint hypothesis. Namely, experiments demonstrate a tendency to produce shoulder-elbow movements by rotating one (leading) joint actively and trail the other joint predominantly passively, with interaction and gravitational torques. We propose that the reason for using this trailing joint control pattern is that it reduces NC. This conclusion follows from NC estimation based on Shannon's theory of information, according to which, if a variable x is limited by an interval (xmin, xmax), and the maximum acceptable error in measurement (or production) of x is d, the amount of information required for encoding x value is I = ln(N), where N = (xmax - xmin)/d. We assume that information processing is described by a control law U = F(S), where S is the motor plant's state and U=(U1,⋯, Un) is a vector of control components. The amount of information I = I1 +⋯+ In required for encoding U can significantly depend on control strategy. During shoulder-elbow movements, coordinated contribution of muscle and passive torque to net torque is required, and therefore, U is represented by muscle torque contribution (MTC) to net torque at each joint. The trailing pattern reduces NC estimated as I by imposing low requirements on MTC error (d is high) at the leading joint and minimizing MTC magnitude [(xmax - xmin) is low] at the trailing joint. We hypothesize that NC is a dominant (as compared to muscle energy) cost function component for optimization of joint coordination and that the CNS decreases NC by using the trailing control pattern. We provide ample experimental support for this hypothesis.

Disclosures: N. Dounskaia: None. **Y. Shimansky:** None. **W. Wang:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.06/X23

Topic: D.17. Voluntary Movements

Support: CIHR #MOP-97944

Title: A test of dual drift-diffusion and independent-race models of reach target selection

Authors: *S. DUROCHER, J. BEAULAC, L. AIT ALI, J. F. KALASKA;
Neurosciences, Univ. De Montréal, Montreal, QC, Canada

Abstract: It is widely accepted that 2-choice decisions result from accumulation of net evidence favoring one choice over the other, but this assumption has rarely been tested rigorously with a range of stimuli that independently vary both the total evidence for each choice and the net evidence for one. Two studies with appropriate ranges of RDK stimuli support net evidence accumulation (Niwa & Ditterich 2008; Lam & Kalaska 2012). In contrast, a study with pairs of stimuli with many different levels of brightness supports a race model with 2 accumulators that each integrates the brightness evidence for their preferred choice (Liston & Stone 2013). Their critical finding was that the RTs for both chosen and rejected stimuli were positively correlated with the brightness of the corresponding stimuli. A net evidence model would predict RTs that are oppositely correlated to the brightness of the chosen and rejected stimuli. Given the fundamental importance of how sensory evidence is processed to reach a decision, it is essential to test this question across a wide range of task conditions. Coallier & Kalaska (2014, 2015) used a "choose and go" (CG) task in which subjects chose between two colored reach targets by deciding whether a multi-colored checkerboard-like Decision Cue (DC) contained more squares of the color of one target. Subjects' responses to a limited set of 10 DCs depended mainly on the difference in the numbers of squares of the 2 target colors. We tested this result more thoroughly with a set of 120 DCs with a wide range of total and relative numbers of colored squares, ranging from DCs with 30/0-100/0 squares of the two colors (evidence for only one target) to DCs with 30/30-100/100 squares (equal evidence for both targets). Five subjects each performed >6000 trials of the CG task over several sessions. Across all DCs, RTs were positively correlated with the total number of squares for the chosen target (mean r=+0.28, range +0.17 to +0.32) and negatively correlated with the number of squares for the rejected target (mean r = -0.46, range -0.23 to -0.61). Testing only DCs with squares of both target colors, positive correlations for chosen targets increased (mean r = +0.42, range +0.21 to +0.56) and correlations for rejected targets remained negative (mean r = -0.16, range -0.06 to -0.21). RTs for DCs with 0 net evidence (30/30 to 100/100) were uniformly long and weakly correlated to the numbers of squares (mean r = 0.04, range 0.12 to -0.05). The results suggest that subjects' decisions were strongly influenced by the difference in the numbers of task-relevant squares in the DCs, rather than a race between accumulators driven by the total number of colored squares supporting each target.

Disclosures: **S. Durocher:** None. **J. Beaulac:** None. **L. Ait Ali:** None. **J.F. Kalaska:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.07/X24

Topic: D.17. Voluntary Movements

Support: NIH R01 HD065438

Title: Effects of movement duration on use of the affected limb in individuals post-stroke

Authors: *S. KIM[1], H. PARK[1], C. E. HAN[2], C. J. WINSTEIN[1], N. SCHWEIGHOFER[1]; [1]USC, Los Angeles, CA; [2]Korea Univ., Seoul, Korea, Republic of

Abstract: Patients with stroke often exhibit non-use of their more affected arm: Although capable of generating arm movements, they often chose not to. Here, we hypothesize that the slowness of movements with the more affected side is a significant factor underlying such non-use. Twelve individuals with chronic stroke (mild to moderate impairment; 46.1± 9.0 on Fugl-Meyer assessment) and six age-matched non-disabled participants performed the Bilateral Arm Reaching Test (BART; Han, Kim, et al. 2014) in the forced and free choice conditions with three different movement time constraints: no time constraint, medium (~1000 ms, depending on target location) and fast (~ 500 ms). Arm kinematics, movement time, and task success were recorded across conditions and amount of use in the free choice condition was computed and compared to normative data. Whereas the non-disabled group showed no differences in hand choice across conditions, the stroke group showed decreased affected hand use in the faster conditions. Further, individuals with left hemiplegia showed a dramatic decrease in use in the fast condition (68% decrease in paretic limb use compared to no time constraint condition), whereas individuals with right hemiplegia showed a only a 24% decrease in paretic limb use. We discuss our results in lights of the known right/left hand differences in arm control and in the framework of delayed rewards discounting.

Disclosures: S. Kim: None. **H. Park:** None. **C.E. Han:** None. **C.J. Winstein:** None. **N. Schweighofer:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.08/X25

Topic: D.17. Voluntary Movements

Title: Challenging the modular control hypothesis: evidence for a functional space-by-time decomposition underlying the muscle activity of whole-body movements

Authors: ***P. HILT**[1], I. DELIS[2], T. POZZO[1,3], B. BERRET[4];
[1]INSERMU1093, Dijon, France; [2]Inst. of Neurosci. and Psychology, Glasgow, United Kingdom;
[3]Istitutio Italiano Di Tecnologia, Genova, Italy; [4]CIAMS, Paris, France

Abstract: Many studies have provided evidence that the muscle patterns underlying a wide variety of motor tasks can be approximated by combinations of a small number of invariant elements, also referred to as muscle synergies. In this study, we aimed at challenging the modular control hypothesis by using a very large data set with many motor tasks, many repetitions per task and bilateral muscle recording during 3D whole-body pointing movements. We asked 4 subjects to perform whole-body point-to-point movements in various directions when standing. The experimental protocol specified 9 targets on 3 vertical bars and subjects had to perform pointing movements between all pairs of targets (i.e. a total of 72 different pointing movements). Participants were asked to repeat each distinct task 15 times, for a total of 2160 movements recorded per participant. We recorded 30 muscles distributed on the whole body, and 22 retro reflective markers to track the motion of anatomical landmarks. To identify putative modules, we used a module extraction method that takes into account concurrently the spatial and temporal dimensions (space-by-time decomposition, Delis et al, 2014). To critically test the extracted modular decompositions, we examined whether tasks can be reliably inferred usinga single-trial decodingmethod described in a previous paper (single-trial task decoding approach, Delis et al, 2013). Overall, our results demonstrated that the 4subjects used a small number of modules (S1(4,4), S2 (6,4), S3(7,4), S4(5,4), spatial, temporalrespectively for each subject) to accomplish all 72 whole body pointing tasks. The extracted space-by-time decompositions consisted of highly similar temporal modules across subjects (average correlation coefficient between temporal modules across subjects: R=0.95) and slightly more variable spatial modules (R=0.6). Importantly, these representations not only accounted for the recorded muscle activity but also allowed highly reliable categorization of the executed tasks. Additionally, the modular structure was found to be especially relevant for task discrimination as compared to alternative methods that used the same number of task-dependent parameters but did not exploit the underlying modularity. In conclusion, we found a low-dimensional representation of muscle activity consisting of functional and consistent spatial and temporal modules to describe reliably a complex high-dimensional set of motor tasks.Thisfinding provides indirect yet strong evidence for the modular control hypothesis and suggests that motor modularity may rely on a separate but concurrent representation in the spatial and temporal domains.

Disclosures: **P. Hilt:** None. **I. Delis:** None. **T. Pozzo:** None. **B. Berret:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.09/X26

Topic: D.17. Voluntary Movements

Title: Influence of chronic stroke on functional arm reaching: Quantifying deficits in the ipsilesional arm

Authors: *R. VARGHESE[1], S. SUBRAMANIAM[2], T. BHATT[3];
[1]Univ. of Illinois At Chicago, Chicago, IL; [2]Univ. of Illinois at Chicago, University of Illinois at Chicago, IL; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: ***Background***: Recent literature has found that following a stroke there may be considerable deficits in the motor control of the ipsilesional upper extremity. These deficits include impairments not only in movement execution, but movement planning as well, resulting in poorer overall quality of movement in the less-affected arm. For example, stroke-related postural problems will tend to further impact postural action preparation that precedes arm movements. Although past studies have evaluated seated reaching tasks, these tasks do not necessarily warrant postural preparation and movement planning as much as a stand-reaching task. Given that reaching while standing is relatively more functional in nature than seated reaching and used extensively in day-to-day activities, it may be valuable to study the impact of stroke on the performance of such a task. ***Purpose***: The purpose of this study was to quantify and compare reaching performance (kinematics and kinetics) of the ipsilesional arm in stroke survivors and age-similar healthy adults, using a reliable stand-reaching paradigm. ***Method***: Community dwelling chronic stroke survivors (n=10) and age-similar adults (n=10) performed two trials of flexion- and abduction-reaching tasks. Surface EMG and acceleration were sampled using wireless sensors from the prime movers, anterior and middle deltoid. Independent t-tests were performed between the two groups to compare response variables that were classified into performance-outcome (reaction time, burst duration and movement time) and performance-production (normalized EMG amplitude and peak acceleration) measures. ***Results***: Individuals with chronic stroke demonstrated a significant delay in performance outcomes (i.e. longer reaction time, burst duration and movement time) compared to their healthy counter parts ($p < 0.05$) for both flexion- and abduction- reaching movements. Likewise, they also exhibited poorer performance production ability (i.e. lower burst amplitude and smaller peak acceleration) ($p < 0.05$) in both reaching directions. ***Conclusion:*** Our results offers quantitative evidence that chronic stroke survivors demonstrate decreased arm movement control in both time (performance-outcome) and amplitude (performance-production) domains than age-similar adults while performing a relatively challenging, but considerably functional, stand reaching task. This may be suggestive of both planning- and execution-related deficits in ipsilesional motor function after stroke. Findings may have critical implications for rehabilitation, especially for interventions employing the ipsilesional arm for compensatory training purposes.

Disclosures: **R. Varghese:** None. **S. Subramaniam:** None. **T. Bhatt:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.10/X27

Topic: D.17. Voluntary Movements

Support: NIH Grant MH099903

NIH Grant NS073952

Title: Cortical representation of bimanual movements: patterns of interference

Authors: *P. THOMPSON, M. LEBEDEV, M. A. L. NICOLELIS;
Duke Univ., Durham, NC

Abstract: Independent movements by two arms, such as reaching simultaneously to two different targets, are prone to patterns of interference. For example, it is easier to move two arms in the same direction than to make different movements with the right and left arm. The underlying neural mechanisms are poorly understood. Here we show that cortical neuronal ensembles in the primary motor cortex (M1) and supplementary motor area (SMA) exhibit distinct patterns of activity that represent patterns of interference during bimanual reaching movements. Rhesus monkeys performed a bimanual joystick centerout task with four possible target locations for each hand. We found that clear patterns of movement interference existed during the task, and this was dependent on target configuration. Using bilateral, multielectrode implants, we recorded from several hundred neurons in M1 and SMA. We observed enhanced ensemble modulations during bimanual versus unimanual movements. Moreover, the degree of bimanual interference had a clear effect on neuronal patterns, particularly in SMA, where neurons were modulated more for more dissimilar movements with two arms. While the movements of each arm were represented by both cortical hemispheres, the distribution of activity across the hemispheres reflected the interference, as well. We suggest that both coordinated and independent bimanual movements are represented in a highly distributed fashion by cortical ensembles. This dynamic and highly plastic representation underlies what is usually called "cortical body schema".

Disclosures: P. Thompson: None. **M. Lebedev:** None. **M.A.L. Nicolelis:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.11/X28

Topic: D.17. Voluntary Movements

Support: R01 NS045853

Title: Characterizing movement initiation in primate motor cortex

Authors: *M. BEST[1], A. J. SUMINSKI[2], K. TAKAHASHI[1], N. G. HATSOPOULOS[1]; [1]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Dept. of Electrical Engin. and Computer Sci., Milwaukee Sch. of Engin., Milwaukee, WI

Abstract: Large ensembles of primary motor cortical (MI) neurons become activated when voluntary arm movements are initiated. The temporal dynamics of this activity have been studied extensively at multiple physiological scales including single cells, small ensembles, and in aggregate via the local field potential (LFP). And yet, how these temporal dynamics vary spatially across the cortical surface remains unknown. Here, we used 96-channel Utah multielectrode arrays to record unit spiking activity and LFPs from four rhesus macaques while they performed an instructed-delay, center-out reaching task. We identified a spatiotemporal sequence of neural activity that preceded movement onset. This spatiotemporal sequence propagated along the rostro-caudal axis of motor cortex and was reflected in unit spiking activity as well as in the beta frequency range (15-30 Hz) of the LFP. This spatiotemporal sequence was absent during motor planning, an epoch when the motor cortex is known to be transiently activated. The causal significance of this phenomenon has begun to be probed using spatiotemporal patterns of intracortical microstimulation. We speculate that movement initiation requires a spatiotemporal recruitment order in MI.

Disclosures: M. Best: None. **A.J. Suminski:** None. **K. Takahashi:** None. **N.G. Hatsopoulos:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.12/X29

Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI 22830086

JSPS KAKENHI 08J09883

CREST JST

Title: The pre-dorsal premotor cortex (pre-PMd), dorsal premotor cortex (PMd), and primary motor cortex (M1) are differently involved in goal-directed behavior based on conditional visuomotor association

Authors: *Y. NAKAYAMA[1,2], T. YAMAGATA[1,2], J. TANJI[2,3], E. HOSHI[1,2,4];
[1]Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Tamagawa Univ. Brain Sci. Inst., Tokyo, Japan; [3]Tohoku Univ. Brain Sci. Ctr., Sendai, Japan; [4]CREST, JST, Tokyo, Japan

Abstract: The dorsal premotor cortex residing in the dorsolateral aspect of area 6 is a rostrocaudally-elongated area, rostral to the primary motor cortex (M1) and caudal to the prefrontal cortex, and is thought to play a central role in the conditional visuomotor behavior. Functional and anatomical studies have revealed that the dorsal premotor cortex is subdivided into its rostral (pre-PMd, area F7) and caudal (PMd, area F2) subdivisions. In the present study, we attempted to examine functional specializations in the pre-PMd, PMd, and M1 with respect to a conditional visuomotor behavior. We examined neuronal activity while monkeys (*Macaca fuscata*) performed a conditional visuomotor task designed to include separate processes for the determination of a behavioral goal (selecting right or left of two potential targets) based on visual-object instructions, action specification based on the goal, and preparation and execution of an action. We found that pre-PMd and PMd neurons retrieve and maintain the behavioral-goal information without encoding the visual object features and, subsequently, specify the actions by multiplexing information for the goal with the spatial-target information. We further found that neurons in the PMd and M1 play a major role in representing the action during the periods of motor preparation and execution, whereas the contribution of the pre-PMd becomes progressively smaller toward the onset of movement execution. These findings revealed that the multiple processing stages underlying the conditional visuomotor behavior are implemented in an area-specific manner as a functional gradient from the pre-PMd to M1, with PMd intervening in between.

Disclosures: **Y. Nakayama:** None. **T. Yamagata:** None. **J. Tanji:** None. **E. Hoshi:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.13/X30

Topic: D.17. Voluntary Movements

Support: ASPIRE Grant 11530-13-33191

Magellan Grant 11530-13-33134

ASPIRE Grant 11530-15-38819

Title: Deficits in visual search following stroke contribute to impaired visuomotor processing and executive function

Authors: ***T. SINGH**[1], C. PERRY[1], A. ROSS[1], J. FRIDRIKSSON[2], S. FRITZ[1], T. M. HERTER[1];
[1]Exercise Sci., [2]Communication Sci. & Disorders, Univ. of South Carolina, Columbia, SC

Abstract: Introduction: Many activities of daily living, such as driving, require coordination of eye and limb movements to gather visual information and act on the environment. Executive function (mental processes that organize and regulate perception, cognition and action) is responsible for integrating visual information, working memory, and prior knowledge to organize eye movements (visual search) and limb movements (motor behavior). We know that visual search and motor behavior are commonly impaired following stroke, but our understanding of how impairments of visual search contribute to deficits in motor behavior is limited. Here we use a robotic version of a common neuropsychological assessment, the Trail Making Test (TMT), to examine the extent to which impairments of visual search contribute to deficits in visuomotor processing and executive function after stroke. Methods: We used the KINARM Endpoint Lab with integrated gaze tracking to examine 69 adults in 3 groups: Young (21-50 yrs, n = 37), Older (52-77, n=16), and Stroke Survivors (48-85, n=16). Subjects used the robotic device to perform the TMT, which involve connecting numbers (TMT-A: 1, 2, 3.), or numbers and letters (TMT-B: 1, A, 2, B.) in the shortest possible time. To minimize the contribution of motor impairments, controls performed the test with their dominant hand and stroke survivors with their less affected hand. We measured the total time (Total Time) to complete the test and the number of reaching errors. Total Time was further divided into the time spent when the hand stayed on a number/letter (Dwell Time) and moved between them (Movement Time). From the eye-tracking data, we measured the number of saccades and fixation duration at each number/letter. Results: Total Time increased progressively from young to older to stroke survivors. The numbers of errors were higher in TMT-B than in TMT-A. The average Dwell Time and Movement Time were longer for TMT-B than TMT-A and also increased progressively between the three groups. The count of saccades to numbers/letters for TMT-B were about ~63% higher than for TMT-A. Stroke survivors made more number of saccades and fixated longer at each number/letter than the healthy controls. During both the dwell and movement phases, stroke survivors made more saccades than the young and older controls. Conclusions: These results indicate that impairments of visual search contribute to decreases in TMT performance. Based on our results, we created a stochastic model of visual search that showed that the main determinant of deteriorated TMT performance in stroke survivors was their inability to combine working memory with topographic strategies during visual search.

Disclosures: T. Singh: None. **C. Perry:** None. **A. Ross:** None. **J. Fridriksson:** None. **S. Fritz:** None. **T.M. Herter:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.14/X31

Topic: D.17. Voluntary Movements

Support: NIH Grant NS035103

Title: Reversible deactivation of motor cortex reveals functional connectivity with anterior and posterior parietal cortex in Old World monkeys (Macaca mulatta)

Authors: *D. F. COOKE[1,2], A. B. GOLDRING[1,2], M. K. L. BALDWIN[1], M. S. DONALDSON[1], L. KRUBITZER[1,2];
[1]Ctr. Neurosci, [2]Psychology, UC Davis, Davis, CA

Abstract: A complex network of connections between motor (M1), premotor (PM) and posterior parietal cortex (PPC) in primates plays an important role in voluntary movement. Long-train intracortical stimulation (ICMS) in all of these fields elicits complex movements, which are segregated into domains of ethologically relevant behaviors such as grasping, reaching or defense. Additionally, M1, PM and portions of PPC send direct projections to the spinal cord in primates. Recently, deactivation of M1 in prosimian galagos and tree shrews (a close primate cousin) has been shown to alter the movements evoked by stimulation of PPC, suggesting that inputs from M1 strongly modulate neurons in PPC. Deactivation of M1 generally affects movements evoked by stimulation in matched PPC domains, although in galagos there appear to be cross-domain effects - interactions between mismatched domains and even mismatched body parts to a degree not seen in tree shrews. For example, deactivating a specific domain in M1 (e.g. forelimb lift) resulted in loss of evoked movement in a different movement domain in PPCr (e.g. hand-to-mouth or eye-blink). The goal of the present investigation was to rapidly and reversibly deactivate forelimb (e.g. grasp) and orofacial domains (e.g. grimace) of M1 by cooling, and to examine the effect of such deactivation on movements evoked from parietal areas (area 1/2, area 5, area 7b) in anesthetized rhesus macaques. By using small (6.9 mm^2) microfluidic thermal regulators we could control both the spatial extent and duration of cooling deactivation and make minute-by-minute observations of the resulting effect. We demonstrate that deactivation of an M1 forelimb domain decreases the amplitude of or eliminates movements evoked by ICMS of matching domains in parietal cortex. We also observed effects on distinct but related movements as well as on very different movements involving other body parts. Such cross-domain effects observed in this investigation may be mediated by the dense intrinsic connectivity in PPC and divergent and convergent reciprocal connectivity between parietal and motor cortex. Through these connections, the activity of an M1 domain greatly impacts both related and unrelated parietal domains. Such functional connectivity of disparate movement representations may underlie coding of movement sequences or, through simultaneous activation, may produce a large number of possible combinatorial movements.

Disclosures: **D.F. Cooke:** None. **A.B. Goldring:** None. **M.K.L. Baldwin:** None. **M.S. Donaldson:** None. **L. Krubitzer:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.15/X32

Topic: D.17. Voluntary Movements

Title: Defining the contribution of the rubrospinal tract to forelimb behavior in rats

Authors: ***K. M. KEEFE**[1], I. SHEIKH[2], C. ENEANYA[2], G. SMITH[2];
[1]Neurosci., [2]Shriner's Pediatric Res. Ctr., Temple Univ., Philadelphia, PA

Abstract: The rubrospinal tract (RST) is an indirect neuronal pathway that contributes to the control of upper arm flexion in humans. Studies in primates show that when the corticospinal tract is damaged, compensatory circuits can form with the RST, allowing for recovery of gross movements of reaching and grasping. Though anatomically well-defined, the RST's involvement in behavioral function in the rat is not fully understood. This study focuses on the discovery of rubrospinal tract impact on forelimb function in a normally behaving rat. We use an inducible dual-vector system featuring the highly efficient retrograde gene transfer (HiRet) lentivirus combined with an adeno-associated TetOn virus (AAV-TetOn) and injection of doxycycline to induce synaptic shutdown of RST neurons in these animals. HiRet is injected unilaterally into the C5-C7 spinal cord to target RST synapses in the area of forelimb motor pools, and TetOn is injected into the contralateral red nucleus to target RST neurons. Doubly infected cells are unable to release neurotransmitter upon application of doxycycline. Behavioral measures include forepaw placement during exploratory rearing, food manipulation and digit movement assessment via the IBB scale, strength of forepaw grip, and coordination of forelimb, shoulder, wrist and paw pronation when reaching for a food pellet. We found that animals injected with this system have significant forelimb deficits on the treated side in the presence of doxycycline. These deficits illustrate the specific involvement of RST neurons in forelimb function.

Disclosures: **K.M. Keefe:** None. **I. Sheikh:** None. **C. Eneanya:** None. **G. Smith:** None.

Poster

342. Reach Control: Selection Mechanisms

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 342.16/X33

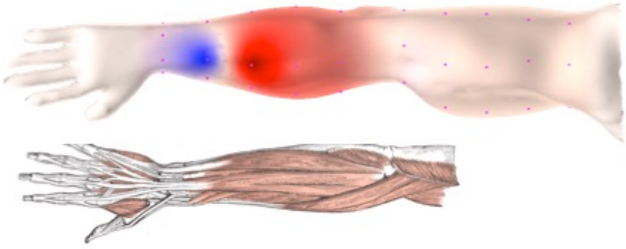
Topic: D.17. Voluntary Movements

Support: The Swartz Foundation

Title: Relationships between high-density EEG and arm EMG dynamics

Authors: *S. MAKEIG, L. PION-TONACHINI;
Inst. of Neural Computation, UCSD/INC/SCCN, La Jolla, CA

Abstract: Electromyography (EMG) records electrical potentials created by muscle activity either using surface electrodes placed on the skin (sEMG) or needle electrodes inserted into a muscle body (iEMG). sEMG is typically assumed to assess the activities of superficial muscles only and is adversely affected by fatty tissue, while iEMG is invasive and requires expert knowledge (and some subject fortitude) to use safely. Both modalities suffer "crosstalk," the instantaneous linear mixing of volume-conducted electrical potentials that also degrades interpretation of both scalp and intracranial electroencephalographic data. In both EEG and EMG records, therefore, individual electrode channel signals sum the volume-projected activities of many spatially distinct brain and/or nearby muscle sources. Independent Component Analysis (ICA) can separate high-density EEG data into activities consistent with activity in a single cortical patch, and can also be meaningfully applied to EMG. Here we performed a pilot study using concurrent high-density whole-arm EMG and whole-head EEG recording to test a new way to analyze non-invasively recorded sEMG in which ICA decomposition of unrectified sEMG data learns a set of spatial filters that estimate the activity time courses and spatial projection patterns of spatially distinct and temporally maximally independent muscle sources. The spatial projection patterns were generally compatible with dipolar sources oriented parallel to and located at one or both ends of a single muscle, as expected from electrical modeling of muscle activity. Pairwise mutual information, computed between the effective muscle sources, showed that sources with overlapping arm surface projections (and, likely, adjacent muscle tissue sources) exhibited stronger interdependency. By exploiting the wealth of methods already developed for EEG analysis, source-resolved EEG/EMG analysis, should allow separation of specific muscle activities and more detailed analysis of EMG-EEG source interactions, while avoiding the need for invasive needle electrode recordings.



Disclosures: **S. Makeig:** None. **L. Pion-Tonachini:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.01/X34

Topic: D.17. Voluntary Movements

Support: HKU 7460/13H

HKU 7482/12H

Title: Shared neural sensory signals for eye-hand coordination in humans

Authors: *L. LI[1], D. NIEHORSTER[1], L. NI[1], D. LISTON[2,3], L. STONE[2];
[1]The Univ. of Hong Kong, Pokfulam, Hong Kong; [2]NASA Ames Res. Ctr., Moffett Field, CA;
[3]San Jose State Univ., San Jose, CA

Abstract: Previous studies that examined eye and hand tracking using either a self-driven or a predictable moving target proposed that eye-hand coordination is realized by a coordination control system that uses both efferent and afferent information of hand movement to synchronize and couple eye and hand motor systems. In the current study, we examined the correlation between the noises of eye and hand tracking of an unpredictable moving target that made efferent and afferent information of hand movement unusable. In Experiment 1, two experimental conditions were tested: in the eye-hand condition, while participants used their eyes to track the movement of a Gaussian target (σ=0.6 deg) on a computer display (40 deg H x 30 deg V) as its horizontal position was perturbed by the sum of seven harmonically-unrelated sinusoids (0.1-2.19 Hz), they also used their dominant hand to control the horizontal position of a second vertically-offset Gaussian cursor (8 deg below) with a high-precision mouse to align it with the target. In the eye-alone condition, the target and cursor positions previously recorded in the eye-hand condition were played back, and participants were instructed to use only their eyes to track the movement of the target. In Experiment 2, the same two experimental conditions were tested except that there was no cursor to indicate hand tracking on the screen. Prior to computing the correlation between the noises of eye and hand tracking responses, for each 90-s trial, we subtracted best-fitting linear tracking responses at the seven input perturbation frequencies to remove direct correlation with the visual stimulus. For both experiments, trial-by-trial examination revealed that the correlation between the residual noises in eye and hand tracking in the eye-hand condition was both highly significant and much higher than the spurious correlation found when eye tracking was not accompanied by simultaneous hand tracking in the eye-alone condition. Our study provides the first behavioral evidence showing that common neural sensory signals drive both eye and hand motor systems and limit eye-hand coordination. This is consistent with the neurophysiological findings of common neural substrates that serve both eye and hand tracking.

Disclosures: L. Li: None. **D. Niehorster:** None. **L. Ni:** None. **D. Liston:** None. **L. Stone:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.02/X35

Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI Grant #26120003(to T.I.)

JSPS KAKENHI Grant #13380602(to G.G.))

Title: Outcome prediction of observed actions affects one's own outcome estimation but not action correction

Authors: *T. IKEGAMI[1], G. GANESH[2];
[1]Natl. Inst. of Information and Communications Technol., Suita City, Osaka, Japan; [2]CNRS-AIST JRL(Joint Robotics Laboratory), UMI3218/CRT, Intelligent Systems Res. Institute,, Natl. Inst. of Advanced Industrial Sci. and Technology(AIST), Ibaraki, Japan

Abstract: The ability to estimate and predict outcomes of self-generated actions and actions observed in others is fundamental for our social life. Previous motor studies have shown that estimation of self-generated actions is achieved by forward models. However, whether the same forward models that help us estimate the outcomes of our own actions are also used to predict the outcomes of actions observed in others remains controversial. Furthermore the structure of the forward model in the action prediction architecture has been a source of recent debate. The traditional view from motor control believes the core role of the forward model to be sensory estimation but not motor command generation, which in turn is handled by the Inverse model. On the hand, some recent models propose a single forward model to play an integrated role and contribute to both action prediction and motor command generation and thus regard a distinct inverse model as dispensable. The goal of this study is two-fold; to examine if a same forward model is involved in both observed outcome prediction and self outcome estimation by humans, and to examine whether the forward model is distinct from the inverse model in the brain. First, we use our novel observation learning paradigm (Ikegami & Ganesh, 2014) to show that a change induced in prediction ability of observed actions by a subject affects the subject's ability to self-estimate the outcome of his own actions. Next, we use model based analysis to distinguish and quantify the changes in the outcome forward model in our experiment. This enabled the critical exhibition that the changes in the subject's self-estimation accuracy were not due to errors in the input (i.e. efference copy of motor command) to the outcome forward model but due to changes in the outcome forward model itself. Our results thus exhibit a causal relation between change in outcome prediction of observed action and the change in one's own outcome forward model. Finally, we exhibit that while the outcome forward model of subjects changed with their outcome prediction ability of observed actions through our task, their outcome inverse

model remained unaffected, exhibiting a distinction between the forward and inverse model in the brain at least at the level of outcome prediction.

Disclosures: **T. Ikegami:** None. **G. Ganesh:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.03/X36

Topic: D.17. Voluntary Movements

Support: HRB Grant: CSA/2012/(201805 / 12584)

Title: Investigation of head and eye movements using the Oculus Riff and electrooculography

Authors: ***B. QUINLIVAN**[1], J. BUTLER[1], I. BIESER[2], N. FOLEY[1], M. HUTCHINSON[2], R. REILLY[1];
[1]Trinity Col. Dublin, Dublin, Ireland; [2]St. Vincent's Univ. Hosp., Dublin, Ireland

Abstract: It is well documented that the appearance of visual targets outside the central field of view usually elicit an eye saccade and a head movement resulting in an overall change in gaze. While saccades have been well characterized through electrooculography and eye tracking, head movements have been less widely investigated. This may be due, in part, to the relative difficultly involved in concurrently recording head and eye movements. Studies that have looked at head movements generally rely on motion capture systems and separate external stimulus such as eccentrically located LEDs. While these set-ups have produce robust and accurate objective measures of head movement, they also come with a number of unavoidable drawbacks. Motion capture systems require large open spaces, lack portability, are prohibitively expensive to smaller studies and are limited by the choice of stimulus and mode of stimulus presentation. Here, we utilize the capabilities of a Head Mounted Display (Oculus Rift Developer kit 2) to study head movements in a Posner paradigm that address many of the limitations of those paradigms built around motion capture systems. The Head Mounted Display is capable of simultaneously presenting an immersive 3D environment with precision while also collecting head turn trajectories in 3 axis (yaw, pitch and roll) at up to ~1000Hz. 15 participants (18-25yrs) performed a speeded head turn task while head rotations and eye movements were monitored. Participants were seated on a fixed upright chair in comfortable position. At the start of each trial, participants aligned their heads to a neutral central position and were presented with an arrow cue, indicating the direction of the target. Participants rotated and aligned to the target. The target disappeared after 1.2 seconds, and participants self-initiated a return movement, to the neutral central position. The cue indicated the valid target direction 80% of the time and invalid direction the remaining 20%. The results of this study indicate that participants take longer to

initiate movement for invalid trials. Furthermore, the speed and latency of the movements in the valid trials were highly similar to that previously reported in the literature using similar paradigms. Furthermore, these paradigms have shown abnormal head control in small groups of people with movement disorders.This portable set-up and paradigm holds particular promise for acquiring large normative datasets from controls for comparison with people with movement disorders, such as cervical dystonia.

Disclosures: B. Quinlivan: None. **J. Butler:** None. **I. Bieser:** None. **N. Foley:** None. **M. Hutchinson:** None. **R. Reilly:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.04/X37

Topic: D.17. Voluntary Movements

Support: Tarson Fund

NSF MOTO-IGERT

Title: Design of an experimental platform for voluntary behavioral training and neurophysiological study of the common marmoset

Authors: *J. WALKER, N. HATSOPOULOS;
Committee on Computat. Neurosci., Univ. of Chicago, Chicago, IL

Abstract: The common marmoset is a promising non-human primate model species for behavioral and systems neuroscience. As is true of other non-human primates, it has a well-developed frontal cortex and has remarkable capacities for motor control and sensorimotor integration. Its lissencephalic cortex, small size, and rapid breeding cycle provide important advantages for neurophysiological study including optical imaging, multi-site electrophysiology, and transgenic manipulation. Yet it maintains a reputation for being difficult to train in traditional experimental tasks. We report a novel self-training approach that allows marmosets to voluntarily participate in training for neuroscience experiments studying the cortical contribution to upper limb motor control. We designed a training apparatus to be attached to the home-cage with ducting allowing the marmosets to move to and from the training apparatus at will. We demonstrate that marmosets are able to learn to voluntarily take a posture within the apparatus appropriate for recording of both neural activity and the kinematics of the upper limb, and that marmosets are willing to participate in training throughout their waking hours in a manner similar to that described for their natural foraging behavior in the wild. This work provides a foundation for the use of the common marmoset as a model system for studying the neural basis of upper limb motor control.

Disclosures: **J. Walker:** None. **N. Hatsopoulos:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.05/X38

Topic: D.17. Voluntary Movements

Title: Evaluating motor and cognitive performances of elderly people using their handwriting features

Authors: ***M. TABATA, IV**[1], M. MATSUBARA[1], K. WATANABE[2], T. WATANABE[2], E. TANAKA[2], T. ANME[2], H. KAWAGUCHI[1];
[1]Dept. of Life Sci., Toyo Univ., Gunma, Japan; [2]Univ. of Tsukuba, Ibaraki, Japan

Abstract: We aimed to establish a simple quantitative method for evaluating motor and cognitive performances of elderly people by analyzing their handwriting characteristics using a "digital pen." This device, which digitizes handwriting with a spatial resolution of 0.3 mm and a temporal resolution of 13 ms, can easily and simultaneously acquire data from many users. Therefore, the digital pen is suitable for screening decreasing motor and cognitive performances. The participants were 219 elderly people (56-93 years old, 20 males, 199 females) from the Joso project (Elderly People Health Empowerment Project in Joso city, Ibaraki Prefecture, Japan), which was conducted in co-operation with Joso city, University of Tsukuba, and Toyo University. Participants were required to use the digital pen to draw a picture without lifting the pen from the paper (one-stroke sketch task) and to complete two questionnaires, the Motor Fitness Scale (MFS) and a questionnaire relating to cognitive performance. Their physical and motor functions (PMF), such as body height, body weight, abdominal girth, handgrip strength, timed up and go test (TUG), and skeletal muscle index (SMI) were also measured. We analyzed several handwriting characteristics such as stroke time, writing pressure, writing speed, and writing speed acceleration. Significant correlations were noted between some handwriting indices and the scores of some MFS or PMF parameters (level of significance, $p < 0.05$). We also found significant correlations between MFS scores and those of the questionnaire relating to cognitive performance. Thus, handwriting data could be used in the simple evaluation of the motor and cognitive performances of elderly people, which, in turn may help in developing strategies for maintaining their quality of life. The protocols used in this study were approved by the Ethics Committee at University of Tsukuba.

Disclosures: **M. Tabata:** None. **M. Matsubara:** None. **K. Watanabe:** None. **T. Watanabe:** None. **E. Tanaka:** None. **T. Anme:** None. **H. Kawaguchi:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.06/X39

Topic: D.17. Voluntary Movements

Support: Alana Foundation

CNPQ

Capes

Title: Description of sensitive tactile and motor pattern in infants with down syndrome

Authors: *D. HERRERO;
Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: INTRODUCTION: As important as knowing "what" changes during motor development is to know "how" and "when" these changes occur. The absence of such data prevents us from describing this process in a continuous and linear fashion in groups of individuals with motor dysfunction, such as infants with Down syndrome. For example, the main references to motor development that are still used in published studies only informs us about the duration of reflexes, but not their pattern. Such studies report on movement intensity, but not on how it can be differentiated from the typical pattern. They may also mention that there is a delay on the emergence of gait, but not whether there is a relation between gait and motor memory. These data would give us relevant information on which stage of motor development infants with Down syndrome may have an alteration in the typical pattern of movement. In addition, they would allow us to optimize specific strategies of intervention for this group. PURPOSE: To describe the pattern of motor development in infants with Down syndrome. METHODS: This is a cross sectional study that is being conducted from March to July, 2015, in the Follow-up Ambulatory Unit at Darcy Vargas Children's Hospital, São Paulo, Brazil, in a clinic specialized in the care of infants with Down syndrome. The following steps are being performed: (1) memory assessment, (2) General Movements, and (3) application of questionnaire. The invitation for parents to participate in the research occurs during routine medical follow-up visits. RESULTS: Currently in this study, we have observed infants from birth to five months with diagnosis of Down syndrome presenting spontaneous movements characterized by moderate abnormal speed and range, with abrupt initiation and termination of movement, and uneven motion flow. Regarding the memory assessment, compared with preterm and term infants groups from previous studies, the subjects of our study were more like the preterm group in relation to the timing of activity execution, displaying longer time to recognize an object. With the video of spontaneous movement, we have data indicating that the articular ends of the feet and toes present less flexion in kick movements and proximity to body trunk, which translate into less

muscular tension and movement range. CONCLUSION: Both assessment scales give us information on the effect of trisomy 21 on central nervous system. The unique feature of our study in relation to previous studies is that both assessments are being done simultaneously, which allows us to characterize motor pattern, memory and articular motion and hopefully inform future, more specific intervention to this group.

Disclosures: D. Herrero: None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.07/X40

Topic: D.17. Voluntary Movements

Support: NIH Grant 5KL2TR000126-04

Title: Memory-guided force output is associated with ADHD symptomatology in young adults

Authors: *K. A. NEELY[1], A. YODER[1], A. CHENNAVASIN[1], G. K. R. WILLIAMS[3], C. L. HUANG-POLLOCK[2];
[1]Dept of Kinesiology, [2]Dept of Psychology, Pennsylvania State Univ., State College, PA;
[3]Anglia Ruskin Univ., Cambridge, United Kingdom

Abstract: Visual feedback is a rich source of information for guiding human motor performance and provides a basis for the formation of memory to guide performance in the absence of visual feedback. The goal of this study was twofold: (1) to examine visually and memory-guided precision grip force in young adults and (2) to examine the relationship between motor performance and scores on the Conners' Adult ADHD Rating Scale (CAARS). To create a large group with a range of CAARS scores, we recruited adults ages 18-25 in two categories, those who self-identified as having ADHD persisting into adulthood and those who self-identified as never having been given a diagnosis of ADHD. Participants (N = 40) completed 20-second trials of isometric force with their index finger and thumb, equal to 25 % of their maximum voluntary contraction. The visual display contained a movable force bar and a stationary target bar. Participants were instructed to move the force bar vertically to overlap the target bar indicating the target amplitude. Real-time visual feedback was provided for the first 8 seconds of the trial. In the remaining 12 seconds, participants were to maintain the same amount force in the absence of visual feedback. All participants completed CAARS-Self Report: Short Version and subscale scores were used as covariates. When participants had visual feedback to guide their force output, force was maintained at a constant level in the last 12 seconds of the trial. In other words, there was no effect of time on mean force output when visual feedback was available. In contrast, when visual feedback was removed, force output decreased as a function of time.

Moreover, the rate of decrease was associated with higher scores on the CAARS subscales for Inattention/Memory Problems, Impulsivity/Emotional Lability, and ADHD Index. This finding suggests that these cognitive domains are important for developing an accurate and stable representation from which to guide force production in the absence of visual feedback. Further, cognitive impairment in these domains may contribute to abnormalities in sensorimotor control in young adults with ADHD.

Disclosures: K.A. Neely: None. **A. Yoder:** None. **A. Chennavasin:** None. **G.K.R. Williams:** None. **C.L. Huang-Pollock:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.08/X41

Topic: D.17. Voluntary Movements

Support: ERC Advanced Grant HUMVOL

Title: Transferring experience of agency from voluntary to involuntary movement

Authors: *N. KHALIGHINEJAD, P. HAGGARD;
Univ. Col. London, London, United Kingdom

Abstract: Sense of agency refers to the capacity to control one's actions, and, through them, the external world. Internal signals within the basal-ganglia-thalamocortical loops are associated with a conscious sense of trying, or willing. Association between these signals with other events could be a basic mechanism for acquiring sense of agency. This presumably occurs during early human motor development, but is not remembered. We have investigated whether healthy adults could acquire new agency-like experiences with respect to involuntary movements, through such associative mechanisms. We used the perceived temporal relationship between an action and its sensory outcome as an implicit proxy measure of sense of agency. 34 healthy volunteers, 18-35 years of age were tested in two separate experiments. In the first experiment, self-paced voluntary actions of one hand were paired with involuntary twitches of the other hand, triggered by transcranial magnetic stimulation (TMS), followed by a tone 250ms later. These learning trials alternated with test trials containing only involuntary twitches followed by tones. Participants judged the time of the tone using a rotating clock display: a perceptual shift of tone towards preceding action is an established index of agency. In a control experiment, participants again judged the time of the tone following an involuntary twitch, but the twitch was never associated with any voluntary action. In the first experiment, participants perceived tones as shifted towards the test trials with TMS-induced twitches that caused them. This ‘intentional binding’ was absent in the control experiment. We showed, for the first time, that coupling an

involuntary movement to a voluntary action leads to acquiring an agency-like experience with respect to the involuntary movement. This finding suggests that we learn to be voluntary. This research could guide development of neuroprosthetic systems designed to augment voluntary motor control.

Disclosures: **N. Khalighinejad:** None. **P. Haggard:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.09/X42

Topic: D.17. Voluntary Movements

Title: Capacity of motor representation through the task of implicit motor imagery in patient with mild cognitive impairments

Authors: ***J. BOURRELIER**[1,2], A. KUBICKI[1,2], O. ROUAUD[3], F. MOUREY[1,2];
[1]INSERM U1093 Cognition Action Sensorimotor Plastic, Dijon, France; [2]Univ. of Burgundy, Dijon, France; [3]Memory Resources and Res., Univ. health Ctr., Dijon, France

Abstract: Mild cognitive impairment (MCI) is an early stage associated in Alzheimer's disease (AD). It is characterized by a cognitive decline greater than expected for age without a significant disruption in one's daily functionality (Albert, 2011). The investigations in motor control during early stage of AD are relatively scarce. This topic should raise a greater interest to allow the introduction of preventive action to maintain the functional physical capacity in aged people with cognitive impairments. In the present study, we wanted to assess the mental representation ability in patients with mild cognitive impairment related-AD through implicit motor imagery (IMI) task. Motor imagery is the ability to create a mental simulation of movement without a concomitant execution. We used an implicit task of hand laterality judgement (Parsons, 1987). To judge of the laterality, the participant imagines implicitly moving his own hand into the orientation and the view (e.g. palm) of the stimulus. These motor processes committed in the IMI task, are engaged also at the motor preparation of action and followed the common neurophysiological way (Jeannerod, 2001). 24 elderly participants aged between 65 and 90 years old (12 women, 73.4 ± 6 years, mean ± S.D.), 12 subjects whom have a Mild Cognitive Impairments related to AD (MCI group) and 12 healthy aged adults (HAA group), were included in the study. First, there is no difference of response time (RT) and accuracy (Acc, calculated though the rate of wrong responses) between the both groups on a simple reaction time task (SRT) and a discrimination task (DT) between two neutral stimuli (left or right arrows) (SRT: RT, $t20 = -1.54$, $p=0.138$; DT: RT, $t22 = -1.16$, $p=0.255$; Acc, $t22 = -1.54$ $p=0.137$). Comparatively, there is a significant difference of response time and the accuracy for the IMI task between the groups (RT, $t22 = -4.89$, $p < 0.001$; Acc, $t22 = -2.88$, $p = 0.008$). In fact, the

further analysis in IMI performance, revealed disparities between both groups in the hardest conditions of IMI task. These kinds of conditions correspond to mentally representation of the non-dominant hand (RT, hand (left-right)*group, $F(1.22)= 6.965$, $p= 0.014$; post hoc Tukey's test, RThand*MCI , $p< 0.001$), of complex orientation angles and of non-congruent view related to own posture. To conclude, the results showed that the patients with MCI are always able to achieve the IMI because duration of the mental rotation process is influenced by the stimuli orientations (RT-orientation ($F(7.154)=41.768$, $p<0.001$), which reflect different biomechanical constraints (Sauner, Bestmann, Siebner, & Rothwell, 2006).

Disclosures: J. Bourrelier: None. **A. Kubicki:** None. **O. Rouaud:** None. **F. Mourey:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.10/X43

Topic: D.17. Voluntary Movements

Support: NIA R01 AG031769-01

Title: Immature motor control contributes to deficient reactive driving performance in adolescents

Authors: *C. KIM, H. MOON, N. LODHA, E. CHRISTOU;
Univ. of Florida, Gainesville, FL

Abstract: The highest car accident and fatality rates occur for adolescents around the minimum legal driving age (15 years in the US). Our purpose was to determine whether reactive driving in adolescents is impaired relative to young adults. Six adolescents from 13-16 years old (13.7 ± 1.4 years) and eleven young adults (20.3 ± 2.1 years) performed a simulated reactive driving task and an isolated visuomotor task with the ankle. In the reactive driving simulation task, participants controlled the gas pedal during a visuomotor task and responded to a sudden visual stimulus by moving their foot from the gas pedal to the brake pedal. Their goal was to apply 40 N force on the brake pedal as quickly and as accurately as possible. Gas pedal control was quantified with the gas pedal error. Brake pedal control was quantified with the brake force error and variability. The response to the visual stimulus was quantified with the pre-motor response time (from stimulus onset to tibialis anterior (TA) activity onset) and motor response time (onset of TA activity to onset of the brake pedal force). We quantified an overall reactive driving index by averaging the normalized scores from gas pedal control, pre-motor response time, motor response time, and brake force control. During the isolated visuomotor task, participants tracked a sinusoidal target at a frequency of 0.5 Hz by producing isometric ankle dorsiflexion forces. We quantified ankle force variability. We found that adolescents under 16 years of age exhibited

impaired motor control compared with young adults as quantified by greater force variability during the isolated visuomotor task ($t = 5.2$, $p < 0.001$) and greater brake force variability during the reactive driving task ($t = 4.42$, $p < 0.001$). The overall reactive driving index was also greater in adolescents than young adults ($t = 5.53$, $p < 0.001$) and the score was significantly related to age ($R2 = 0.58$, $p < 0.001$). Finally, the overall reactive driving index score was correlated with both measures of motor output variability (isolated visuomotor task force variability: $R2 = 0.37$, $p = 0.013$; brake force variability: $R2 = 0.61$, $p < 0.001$). In conclusion, these findings suggest that immature motor control in adolescents contributes to deficient reactive driving performance compared with young adults. The motor control deficiency in adolescents could contribute to higher car accident rates independent of other contributing factors, such as distraction.

Disclosures: C. Kim: None. **H. Moon:** None. **N. Lodha:** None. **E. Christou:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.11/X44

Topic: D.17. Voluntary Movements

Support: NIH Grant R01AG041878

Title: The effect of coordinate frame on motor learning in Alzheimer's disease and Parkinson's disease

Authors: K. A. CAULFIELD[1], Y. R. MIYAMOTO[2], J. M. BRETON[3], J. B. BRAYANOV[2], M. A. SMITH[2], *D. PRESS[1];
[1]Dept Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Sch. of Engin. and Applied Sci., Harvard Univ., Cambridge, MA; [3]Neurosci., Univ. of Calif., Berkeley, Berkeley, CA

Abstract: Alzheimer’s disease (AD) causes deficits in performing previously learned motor skills, but its effects on the acquisition of motor skills remains uncertain. Independent cortical networks allow for acquisition of motor skills in an intrinsic, body-based reference frame, as compared to an extrinsic, world-based reference frame. Previous studies did not disambiguate these reference frames, possibly explaining the mixed results. The neuropathology of AD shows substantial atrophy in parietal areas that may be crucial for acquisition of extrinsically-referenced skill, but relative sparing of motor cortical regions likely necessary for the acquisition of intrinsically-referenced skill. We therefore hypothesized that AD participants would display deficits in extrinsically-referenced learning, leading to a shift towards acquiring skill in a more intrinsic reference frame. We recently found that healthy participants learn a visuomotor rotation (VMR) task in a combination of intrinsic and extrinsic space. In this study, we tested AD participants (n=17) and age matched controls (n=20) on this task in which participants conducted

9cm point-to-point reaching arm movements to ±30° VMR. Movements in 10 directions were tested both in trained and novel workspaces. In the novel workspace, the arm posture was rotated by 90°. This change in arm posture resulted in a 90° shift in the correspondence between intrinsic and extrinsic representations of the movement direction. In the novel workspace, this allowed the degree to which the peak generalization shifts away from the trained target to quantify how much the learned adaptation is represented in intrinsic vs extrinsic reference frames; no shift would occur if adaptation were represented in a purely extrinsic frame, since the extrinsic movement direction corresponding to the trained target is maintained. In contrast, a 90° shift in generalization would be expected if adaptation were encoded in a purely intrinsic frame. In the novel workspace, we observed shifts in generalization that were significantly closer to 90° for the AD participants than for the healthy elderly controls (69.9° vs. 52.2°). These data indicate that AD patients have reduced extrinsically-referenced learning abilities, perhaps due to parietal lobe dysfunction, $p = .0012$. Lastly, we plan to test VMR shifts in generalization in Parkinson 's disease (PD) participants. Since PD patients have dysfunction in motor cortical regions that are implicated in the acquisition of intrinsically-referenced skill, PD participants may show deficits in intrinsically-referenced learning.

Disclosures: **K.A. Caulfield:** A. Employment/Salary (full or part-time):; Beth Israel Deaconess Medical Center. **Y.R. Miyamoto:** None. **J.M. Breton:** None. **J.B. Brayanov:** None. **M.A. Smith:** A. Employment/Salary (full or part-time):; Harvard University. **D. Press:** A. Employment/Salary (full or part-time):; Beth Israel Deaconess Medical Center.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.12/X45

Topic: D.17. Voluntary Movements

Title: Inter-subject coherence in frontal pole increases during better group walking

Authors: ***S. IKEDA**[1], T. NOZAWA[1,2], R. YOKOYAMA[3], A. MIYAZAKI[4], Y. SASAKI[4], K. SAKAKI[4], R. KAWASHIMA[1,2,4,5];
[1]SAIRC, IDAC, Tohoku Univ., Sendai, Japan; [2]DUS, IDAC, Tohoku Univ., Sendai, Japan; [3]Sch. of Medicine, Kobe Univ., Kobe, Japan; [4]DFBI, IDAC, Tohoku Univ., Sendai, Japan; [5]DDCN, IDAC, Tohoku Univ., Sendai, Japan

Abstract: A rhythm of sound (70 BPM) is known to promote walking flow during the group walking. This phenomenon can be intuitively understood because pedestrians can keep smooth flow of walking along to the rhythm sound. We had a simple question of how the rhythm sound changes pedestrians' cognitive processes. However, no studies have investigated changes of the cognitive processes during the group walking of the rhythm sound. Our hypothesis was that

inter-subject coherence in the frontal pole increases during better group walking. The frontal pole is known to be associated with a role for predicting the behaviors of others during cooperation action. We investigated the coherence between subjects' signals from the frontal pole while the subjects performed the group walking. The brain activities were measured by ultra-small NIRS that can simultaneously and wirelessly measure multi-subjects' signals. In the group walking, the subjects (24 or 25) were asked to walk on a circle with a radius of two meters, and to walk naturally, keeping smooth flow in mind. As a control condition for the group walking, the subjects performed stepping. The stepping is similar in movement to the group walking but the subjects can perform it without consideration for others' pace. Our experiment, therefore, consists of four conditions (i.e. rhythm walking, no-rhythm walking, rhythm stepping, and no-rhythm stepping). Using wavelet transform coherence which is a method of measuring the coherence between two signals as a function of frequency and time, we calculated inter-subject coherence for each frequency (0.01_0.1 Hz). We, then, calculated ΔW (walking) and ΔS (stepping) which showed the coherence difference between rhythm and no rhythm conditions, and evaluated whetherΔW was statistically larger than ΔS for each frequency. We observed that the walking flow increased in the rhythm condition compared to that in the no-rhythm condition. Furthermore, ΔW was larger than ΔS in frequencies from 0.03 to 0.04 Hz, which was interaction effect between the rhythm sound and the group walking. Our results suggested that rhythm sounds like that used in our experiment might promote inter-subject coherence during cooperation action by a group.

Disclosures: S. Ikeda: None. **T. Nozawa:** None. **R. Yokoyama:** None. **A. Miyazaki:** None. **Y. Sasaki:** None. **K. Sakaki:** None. **R. Kawashima:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.13/X46

Topic: D.17. Voluntary Movements

Title: Hand Blink Reflex modulation during a voluntary movement

Authors: *M. BOVE[1], M. BIGGIO[1], E. FALSINI[1], C. FOSSATARO[2], F. GARBARINI[2], A. BISIO[1];
[1]Univ. of Genoa, Genoa, Italy; [2]Univ. of Turin, Turin, Italy

Abstract: Defensive reflexes are primitive and involuntary motor responses to potentially dangerous stimuli, mediated by fast, subcortical pathways. It has been recently demonstrated that the Hand Blink Reflex (HBR), which is a subcortical response at the brainstem level elicited by the electrical stimulation of the wrist, is modulated by the proximity of the stimulated hand to the face, being dramatically increased when the hand is inside the peripersonal space surrounding the

face. The experimental paradigm used in this previous study was based on evaluating the HBR amplitude during different static positions of the hand. In our opinion, the defensive peripersonal space is not only modulated by the position of the hand but also by the intentional direction of the movement. To this aim we investigated the role of the voluntary movements in modulating this defensive physiological response. Here, we asked whether, on equal hand positions, the intentional direction of the hand movements (either close to the face or far from the face) can modulate the HBR. Electromyographic activity was recorded from the orbicularis oculi bilaterally in three different conditions, in which the subjects were asked a) to stay still ("static hand"), to move their arm close to the face ("far-near" movement direction) or far from the face ("near-far" movement direction). In each condition, the HBR was elicited by delivering electrical stimuli to the median nerve at the wrist in three hand positions, depending on the amplitude of the elbow angle: with the arm frontally extended at ~180° (α1; far from the face); tilted at ~90° (α2; intermediate position); tilted at ~45° (α3; close to the face). We extracted the area under the curve of each HBR average waveforms and we entered the obtained values in a RM-ANOVA with two between factors "condition" (three levels: static, up, down) and "angle" (three levels: α1, α2, α3). Our results showed a significant interaction angle*condition ($p = 0.0003$), suggesting that the same angle amplitude induces different HBR modulation, depending on the condition. In particular, in the "static hand" condition, we replicated the previously described HBR increase in α3 (near-position) with respect to the α1 (far-position). In the "far-near" condition, the HBR increase in α3 was found with respect to both α1 and α2. Crucially, in the "near-far" condition, no difference between the three angles was found. These findings suggest that, when the hand is close to the face but the subject is planning to go down, the motor system through a combination of a feedforward and feedback control is able to anticipate the final state of the movement: the "near" becomes "far".

Disclosures: **M. Bove:** None. **M. Biggio:** None. **E. Falsini:** None. **C. Fossataro:** None. **F. Garbarini:** None. **A. Bisio:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.14/X47

Topic: D.17. Voluntary Movements

Support: FISM 2014/R/5

Title: Kinematic description of handwriting movements during fMRI examination

Authors: ***A. BISIO**[1], L. BONZANO[1], L. PEDULLÀ[1], C. BRUNO[1], A. TACCHINO[2], G. BRICHETTO[2], M. BOVE[1];
[1]Univ. of Genoa, Genova, Italy; [2]Fondazione Italiana Sclerosi Multipla, Genoa, Italy

Abstract: Despite the widespread use of computers, handwriting represents an important form of communication among individuals that is acquired from childhood. Indeed, writing plays a crucial role in school performance, with important implications for motor and cognitive development. Due to neurological diseases as, for instance, multiple sclerosis, handwriting skills can deteriorate till interfering with daily life activities. Writing rehabilitation is one of the target of the occupational therapy treatments, which includes new techniques emphasizing the learning and practice of functional motor skills within a task-specific context. However, due to the low sample of scientific work at disposal, reliable quantitative data on the efficacy at behavioral level of this kind of interventions are lacking, as also it is not clear whether and what are their effects on brain networks. The aim of the present study was to define an evaluation methodology that allows to quantitatively characterize the kinematics of handwriting movements and to correlate the behavioral measures with structural and functional brain architecture using non-conventional magnetic resonance imaging (MRI) techniques. This represents the first part of a project whose ultimate goal is to assess the differences in movement planning and execution of handwriting movements between people affected by multiple sclerosis and healthy age-matched controls. As first step we tested the goodness of kinematics measurements inside the magnetic scanner, during a functional MRI (fMRI) session. To do that we compared the spatiotemporal kinematic data acquired by mean of a MRI-compatible tablet (E.M.S. srl, Bologna) during a fMRI test with those obtained in an ecological condition where the subject was seated at the table and wrote with an ink-jet pen over a paper positioned on the surface of the tablet. Twenty-two healthy participants were enrolled for the study. They were requested to write the Italian sentence “Il sole scalda” (i.e., “The sun warms”) for three times in each experimental condition. Kinematic analysis showed that, although spatiotemporal features (movement duration, amplitude and velocity) of the written trace augmented significantly during fMRI acquisition ($p<0.05$), significant correlations among the kinematic parameters acquired outside and inside the magnetic scanner were obtained (duration $p<0.05$, amplitude $p<0.05$, mean velocity $p<0.01$)) indicating a similar motor behavior in these two conditions. Therefore, we can suggest that this approach to evaluate the kinematic of handwriting during fMRI session provides reliable kinematic measurements.

Disclosures: **A. Bisio:** None. **L. Bonzano:** None. **L. Pedullà:** None. **C. Bruno:** None. **A. Tacchino:** None. **G. Brichetto:** None. **M. Bove:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.15/X48

Topic: D.17. Voluntary Movements

Support: ARC Grant DE120100653

ARC Grant FT120100391

Title: Electric and acoustic stimulation during movement preparation can facilitate movement execution in healthy participants and stroke survivors

Authors: *W. MARINOVIC, S. BRAUER, K. S. HAYWARD, T. CARROLL, S. RIEK;
The Univ. of Queensland, Brisbane, Australia

Abstract: The presentation of unexpected loud acoustic stimuli (LAS) during movement preparation has been used to investigate movement preparation and initiation in healthy participants. Recent studies have shown that LAS can also facilitate movement initiation and execution in chronic stroke survivors, motivating the examination of alternate methods of sensory stimulation that could be used in therapies for movement recovery. Here we sought to determine whether somatosensory (e.g. electric) stimulation could be used to facilitate movement execution and initiation in healthy participants and chronic stroke survivors. In Experiment 1, 15 healthy participants performed an arm supination action in response to a visual go-signal. In some trials, we presented either an auditory (50 ms white-noise, 114dBa) or electric stimulus (2 ms pulse) in synchrony with the presentation of the go-signal. In Experiment 2, 12 healthy participants performed the same task, but we delivered electric stimulation either to the ulnar styloid process or to the short-head of biceps brachii. In Experiment 3, 12 healthy participants attempted to perform finger abduction in synchrony with the predicted time of appearance of the last of a sequence of four flashes on a monitor screen. Occasionally, acoustic, electric or combined stimulation were presented 200 ms prior to the expected time of movement onset. In Experiment 4, five chronic stroke patients and 8 healthy adults performed a reaching task in response to a visual go-signal. Acoustic and electric stimuli were used as in Experiment 1. We found that electric stimulation was as effective as acoustic stimulation in facilitating motor actions in both reaction time and anticipatory timing tasks (Exp. 1 and 3), and that the site of electric stimulation made little difference in facilitating motor actions (Exp. 2). Simultaneous acoustic and electric stimulation caused faster release of anticipatory actions than unimodal stimulation of either modality (Exp. 3). Finally, the responses of both chronic stroke patients and healthy adults were faster and initiated earlier than normal in the presence of both types of sensory stimulation in a functional reaching task (Exp. 4). Our results suggest that electric and acoustic stimulation impact movement initiation and execution through arousal elicited by strong and unexpected sensory stimulation. They also demonstrate that cross-modal stimulation can be useful to boost the facilitatory effects of sensory stimulation on movement execution and initiation. These findings may lead to new avenues for experimental and clinical exploitation of the effects of sensory information on movement execution.

Disclosures: W. Marinovic: None. **S. Brauer:** None. **K.S. Hayward:** None. **T. Carroll:** None. **S. Riek:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.16/Y1

Topic: D.17. Voluntary Movements

Support: NSERC (Canada)

ORF (Canada)

CFI (Canada)

CIHR (Canada)

Title: Investigating the role of mIPS in movement planning using HD-tDCS

Authors: ***S. XU**[1], J. P. GALLIVAN[1], G. BLOHM[1,2,3];
[1]Queen's Univ., Kingston, ON, Canada; [2]Canadian Action and Perception Network (CAPnet), Toronto, ON, Canada; [3]Assn. for Canadian Neuroinformatics and Computat. Neurosci. (CNCN), Kingston, ON, Canada

Abstract: The medial part of the intraparietal sulcus (mIPS) has been traditionally viewed to be involved in the integration of target and effector to generate a motor plan. However, the precise contribution of the mIPS is unclear. Previous transcranial magnetic stimulation studies have suggested a role in performing motor vector calculations based on visual cues of initial hand position as well as in implementing the direction vector (Vesia et al 2010; Davare et al. 2011). Here we used high definition-transcranial direct current stimulation (HD-tDCS) to locally modulate neuronal excitability in a polarity-specific manner in the left mIPS to investigate the role of mIPS in movement planning during visually guided reaching. The left mIPS was functionally localized on an individual-basis using functional magnetic resonance imaging (fMRI) during performance of a center-out interleaved pointing and saccade task. We then used BrainSight to localize mIPS on the scalp of each participant and administered anodal and cathodal stimulation (2mA for 20min; 3cm radius 4x1 electrode placement) over the left mIPS in two separate sessions separated by a week within a randomized, single-blind design. Participants were asked to perform memory-guided reaching movements from one of 2 initial hand positions (5cm left/right of midline) to one of 4 randomly chosen briefly flashed targets (20cm distant, 5cm apart horizontally) while fixating on a straight-ahead cross located on the target line. Each participant completed a minimum of 200 control, stimulation and post-stimulation trials. We examined amplitude errors and direction errors for 3 participants. In general, anodal tDCS (during and post stimulation) resulted in over-reaching compared to pre-stimulation controls while under-reaching was observed during and after cathodal stimulation for both ipsilateral and contralateral reaches, and from all initial hand positions to all targets. Directional error analysis revealed that for all subjects, anodal stimulation induced directional gains, specifically under-reaching to all targets, while cathodal stimulation induced directional shifts, specifically, under-reaching to rightward targets, and over-reaching to leftward targets. These results are consistent with a role of mIPS in reach planning. Direction and amplitude modulations in our experiment

indicate that at the level of mIPS, initial hand position and target position have been integrated into a reach vector code. This validates the use of HD-tDCS as a tool to study the causal role of cortical areas in movement planning.

Disclosures: **S. Xu:** None. **J.P. Gallivan:** None. **G. Blohm:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.17/Y2

Topic: D.17. Voluntary Movements

Support: Bavarian Elit Aid Act (BayEFG)

Title: Neuroanatomical basis of the intentional response deficits associated with unilateral neglect: an rTMS study in healthy subjects

Authors: M. GUTIERREZ-HERRERA[1], S. SAEVARSSON[2], T. HUBER[3], *J. HERMSDORFER[1], W. STADLER[1];
[1]Technische Univ. München, München, Germany; [2]Klinikum Bogenhausen, München, Germany; [3]Klinikum rechts der Isar, München, Germany

Abstract: Studies exploring the neuroanatomical basis of the intentional response deficits (IRD) associated with neglect have reported contrasting findings, with lesion sites ranging from right parietal to subcortical and frontal areas (e.g. Vossel et al, 2010; Ghacibeh et al, 2007; Husain et al, 2000). The present study assessed the occurrence of IRD following the application of repetitive transcranial magnetic stimulation (rTMS) to the right middle frontal (rMFG) and angular (rAG) gyri in healthy subjects. Sixteen participants underwent a 10-Hz rTMS protocol (600 ms duration) while performing a pointing task with the right hand to either of two lateral targets, under two task conditions (i.e. internally guided [IG; pointing side freely selected] vs. externally guided [EG; pointing side spatially cued]) and two visual feedback conditions (i.e. blindfolded vs. sighted). The experiment consisted of three counterbalanced blocks including rMFG stimulation, rAG stimulation and no-TMS (control condition). Three main variables were considered for the analysis (i.e. movement time, reaction times and frequency of leftward vs. rightward IG movements). A repeated measures analysis of variance (ANOVA) conducted on movement times revealed a significant interaction between stimulation site and movement direction, $F(2,32) = 10.55$, $p < .05$, with left directed movements showing longer movement times under parietal stimulation conditions compared to control ones. As to reaction times, a repeated measures ANOVA showed a significant interaction between task condition, movement direction and stimulation site, $F(1.44, 21.69) = 9.95$, $p < .05$. This interaction was given by the presence of longer reaction times for left compared to right directed movements under EG

conditions, when receiving frontal stimulation. Furthermore, a generalized linear model analysis with binary logistic regression conducted on the frequency of leftward vs. rightward IG movements, indicated movement direction to be significantly predicted by the interaction between visual feedback and stimulation site, b= -0.58, Wald $\chi 2(1) = 7.06$, $p < .05$. This interaction pointed to a reduced number of leftward movements under blindfolded conditions compared to sighted ones, when frontal stimulation was applied. Our findings suggest that rMFG and rAG have different roles in the manifestation of IRD, with the former related to planning and initiation difficulties and the latter affecting movement execution. Such evidence would be in line with the idea that neglect is a modular syndrome (Coulthard et al, 2007) and the variability of IRD may depend on the amount of damage to specific nodes in the visuomotor network.

Disclosures: M. Gutierrez-Herrera: None. **S. Saevarsson:** None. **T. Huber:** None. **J. Hermsdorfer:** None. **W. Stadler:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.18/Y3

Topic: D.17. Voluntary Movements

Title: Associative sensorimotor learning of manipulation in humans: role of premotor dorsal area

Authors: *P. J. PARIKH, M. SANTELLO;
Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

Abstract: Humans have an exquisite ability to learn motor behaviors through arbitrary sensory cues. Non-human primate work suggests that dorsal premotor area (PMd) is involved in building associations between arbitrary visual cues and movements. However, the role of PMd in arbitrary sensorimotor learning in humans is not well understood. In this study, we hypothesized that integrity of PMd is necessary for learning a sensorimotor task that required human subjects to manipulate, using two-digit precision grip, an object whose center of mass was cued by arbitrary colors. The manipulation task required participants to grasp and lift an inverted T-shaped grip device while balancing it. The center of mass (CM) of the device was pseudo-randomly changed across trials by adding a 400-g mass at the base of the object (right, center, or left location). Through trial and error, participants were required to learn the association between the arbitrary color cue and CM location, and exert the required torque on the object to balance it over 3 blocks of 20 trials each. For right and left CM locations, participants were required to exert compensatory counterclockwise and clockwise torque, respectively, on the object to balance it. For centered CM, participants were not required to exert any torque on the object. We found that right-handed young adults (n=11) learned to associate arbitrary color cues about CM location to balance the object. Specifically, we found a significantly greater torque error

averaged across trials (avTerr) for block 1 than block 2 ($p < 0.01$), whereas no difference was found between avTerr for blocks 2 and 3. Next, we used continuous theta burst transcranial magnetic stimulation (TMS) to disrupt contralateral PMd on separate group of participants (n=8) before they learned the same task. Following disruption of PMd, participants produced greater avTerr for block 1 as compared to participants that did not receive TMS ($p=0.008$). For block 2, there was a trend toward a statistically significant difference between avTerr produced by the TMS group versus No TMS group ($p=0.056$). No group difference was found on the manipulation performance during block 3. These results indicate that disruption of PMd interfered with participants' ability to associate arbitrary cues with anticipatory control of manipulation. Therefore, our study suggests that contralateral PMd plays an important role during acquisition of a sensorimotor task based on arbitrary visual cues.

Disclosures: P.J. Parikh: None. **M. Santello:** None.

Poster

343. Cortical Planning and Execution: Behaviour

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 343.19/Y4

Topic: D.17. Voluntary Movements

Support: H2020 ENHANCE

AFR 1229297

RPG00022/2012

Title: Sparse encoding of natural "in-the-wild" hand movements

Authors: *A. A. FAISAL[1], A. THOMIK[2];
[1]Imperial Col. London, London, United Kingdom; [2]Imperial Col. London, London, United Kingdom

Abstract: A fundamental problem in neuroscience is to understand how the brain translates a symbolic sequence of action descriptors, or high-level motor intention, into appropriate movement of the limbs. Starting from a large collection of hand movement data collected using an unobtrusive motion capture glove “in-the-wild” (i.e. in daily life) we develop a data-driven approach to seek a model of movement generation capable of capturing both spatial and temporal structure of natural behaviour. Drawing inspiration from findings in the sensory system, we assume that the brain generates hand movements by activating a temporally sparse sequence of sparse eigenmotions. These sparse eigenmotions - building blocks of natural movement - are characterised by very high correlation of the joints involved in the movement. To identify the set of sparse eigenmotions required for a parsimonious representation of the data, we developed a

new dictionary learning approach which determines the number and shape of sparse eigenmotions directly from the data in an unsupervised fashion. We show that the dictionaries of sparse eigenmotions learnt are consistent across subjects with some minor variations, likely due to individuality and differences in activities. We also find that the number of sparse eigenmotions identified in the data grows logarithmically with increasing amounts of data, not unlike the number of different words in an English language text. We thus postulate a "language of natural movement" where the brain composes meaningful behaviour by aligning sparse eigenmotions according to a yet to be determined rule set. In a first step towards understanding these rules, we determine the encoding of our data using the learnt dictionaries. Using orthogonal matching pursuit (OMP), we identify the temporal sequence of sparse eigenmotions required to represent the data in optimal fashion. Analysis of the structure of the encoding is currently ongoing. This work has multiple implications: (1) it offers a potential mechanism for movement generation by the brain which is richer and potentially more natural than previous work relying on dimensionality reduction by principal component analysis. Our sparse eigenmotions may thus reflect behaviour induced by neurons akin to grasp-type specific neurons found in the monkey premotor cortex. (2) The knowledge of the spatiotemporal structure of natural movement may contribute to the design of neural prostheses which are easier to control and contribute to a better quality of life for amputees.

Disclosures: **A.A. Faisal:** None. **A. Thomik:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.01/Y5

Topic: E.01. Neuroendocrine Processes

Support: The INOUE ENRYO Memorial Foundation for Promoting Sciences

Title: Effects of androgen on GnRH3 neurons in slice culture of the tilapia brain

Authors: ***Y. NARITA**[1], A. TSUTIYA[1], T. KANEKO[3], R. OHTANI-KANEKO[1,2];
[1]Grad. Sch. of Life Sci., Toyo Univ., Gunma, Japan; [2]Bio-Nano Electronics Res. Ctr., Toyo Univ., Saitama, Japan; [3]Grad. Sch. of Agr. and Life Sci., Tokyo Univ., Tokyo, Japan

Abstract: Gonadotropin-releasing hormone type III (GnRH3) neurons are reported to control male reproductive behaviors such as nest building and aggressive behaviors in tilapia. In our previous study, we found the sexual dimorphism of GnRH3 neurons in tilapia; males have a greater number of GnRH3 neurons in the terminal nerve (TN) than females. Treatment with 11-ketoteststeron (11-KT) and methyltestosterone, but not with 17β-estradiol (E2), increased the number of GnRH3 neurons in mature females to a level similar to that in males (Kuramochi et

al., 2011). In addition, our recent study revealed that androgen treatment increased proliferating (PCNA-positive) cells and Hu-positive neurons in the female TN. We also found that Hu-positive neurons with 5-bromo-2'-deoxy-uridine (BrdU) labeling were significantly increased after simultaneous injection of 11-KT and BrdU to females. Furthermore, about 20% of increased GnRH3 neurons were labeled with BrdU in females injected simultaneously with 11-KT and BrdU, while the rest of GnRH3 neurons were not labeled with BrdU. These results indicated that androgen induced adult neurogenesis in the female TN and that the androgen-induced increase of GnRH3 neurons in females is mediated partly through adult neurogenesis. The objective of the present study is to know from where new GnRH3 neurons originate after females were treated with 11-KT. Do they originate within the TN, or originate from other brain regions and migrate to the TN? To answer the question, we prepared female brain slices including the TN and cultured them with or without 11-KT. When slices were cultured in the medium containing 11-KT (11-KT-treated slices) or just solvent ethanol (control slices) for three days, GnRH3 neurons were significantly increased in 11-KT-treated slices, compared to those in control slices. However, when slices were cultured in the medium containing 11-KT and either BrdU or 5-ethynyl-2'-deoxyuridine (EdU) for three days, GnRH3 neurons with BrdU- or EdU-labeling in the nucleus were scarcely found in the slices. These results indicated that androgen is able to induce the differentiation of GnRH3 neurons and increase their number in the TN, although the new generation of GnRH3 neurons may not be induced within the TN. Therefore, it was suggested that the newly generated (BrdU-labeled) GnRH3 neurons observed in the TN *in vivo* after 11-KT- and BrdU-injections may arise outside the TN and then migrate to the TN.

Disclosures: Y. Narita: None. **A. Tsutiya:** None. **T. Kaneko:** None. **R. Ohtani-Kaneko:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.02/Y6

Topic: E.01. Neuroendocrine Processes

Support: MNSU Biological Sciences

MNSU Undergraduate Research Center

Title: The effect of steroid hormones on neurogenesis in the green anole lizard brain

Authors: N. A. BOOKER[1], J. SON[1], N. D. HART[1], Y. LEE[1], *R. E. COHEN[2];
[1]Biol. Sci., Minnesota State Univ. Mankato, Mankato, MN; [2]Biol. Sci., Minnesota State University, Mankato, Mankato, MN

Abstract: Gonadal steroid hormones, such as testosterone (T) and its metabolites (dihydrotestosterone, DHT and estradiol, E2), are important for maintaining reproductive behaviors and brain morphology, including the survival of new adult-born neurons. These hormones change dramatically in seasonally breeding animals, including the green anole lizard (Anolis carolinensis). In addition to changing steroid hormone levels, anoles have altered brain morphology and reproductive behaviors in the breeding (BS) and non-breeding seasons. For example, the preoptic area (POA) is larger in volume and the medial amygdala (AMY) has fewer neurons in the BS. These structural changes may be related to seasonal differences in steroid hormone levels. To examine how steroid hormones alter brain morphology, we gonadectomized breeding adult male lizards and inserted subcutaneous capsules containing T, E2, or DHT, or capsules that were left empty. Animals were then injected with bromodeoxyuridine (BrdU) to mark new cells. Brains were collected 25 days after the last BrdU injection and an immunohistochemistry for BrdU and Hu C/D (a neuronal marker) was conducted. We found that new neurons (BrdU/Hu positive cells) were present in the AMY of this species and we are currently counting these cells to determine whether different hormone treatments impact the survival of new neurons in the anole AMY during the BS. In addition, we found that animals treated with androgens had a larger POA volume, as defined by Nissl staining, compared to those treated with empty or estradiol capsules, and are currently investigating the effects of steroid hormone treatment on cell number and soma size in both the AMY and POA. These preliminary results help to further determine how steroid hormones impact changes to brain morphology and neurogenesis during the breeding season in a non-avian reptile.

Disclosures: **N.A. Booker:** None. **J. Son:** None. **N.D. Hart:** None. **Y. Lee:** None. **R.E. Cohen:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.03/Y7

Topic: E.01. Neuroendocrine Processes

Title: The last hours before singing··· short-term dynamic transcriptomes after testosterone treatment in female canaries

Authors: *M.-C. KO[1,2], C. FRANKL-VILCHES[1], A. BAKKER[1], M. GAHR[1];
[1]Max Planck Inst. For Ornithology, Seewiesen, Germany; [2]IMPRS for Organismal Biol., Seewiesen, Germany

Abstract: Singing is a sexually dimorphic behaviour in domestic canary (Serinus canaria), given that female canary song is rarely observed in nature. Nonetheless, female song can be induced within 3-5 days and crystalized (fully differentiated) within 1 month by testosterone (T)

treatment. Such plasticity of female canary provides an excellent model to study hormone modulation in the brain hence stimulation of behavior. To investigate the dynamic expression of T-driven singing-related genes and their biological pathways of the emerging female singing, we analyzed female canaries treated with T for short (1, 3, 8 hours) or long duration (3, 7, 14 days), and untreated controls (n=6 in allgroups). Plasma T concentration and vocalization were monitored before and during implantation. Animals were sacrificed by decapitation after the desired implant duration, and brains were removed. Subsequently, we performed exon-level microarray analyses (Affymetrix) of the transcriptomes of microdissected HVC (a crucial area of the song control system) and entopallium (visual region). We detected singing activity from day 4, whereas the plasma T increased radically (>450 fold than baseline) after 1h, peaked at 3h (>550 fold), and gradually decreased until 14d (>130 fold). Correspondingly, HVC exhibited rapid and strong transcriptional response to T, as 684, 4990 and 4549 genes were up-regulated after 1, 3, and 8 hours, respectively; compared to the untreated controls. Further, we found long-lasting transcriptional responses given that 4022, 2594 and 5946 genes were up-regulated after 3, 7 and 14 days, respectively. Whereas about 18% of genes expressed in HVC were continuously up-regulated between 3h and 2 weeks of T-exposure, about half the transcriptional regulation was dynamic and progressive. Gene ontology analysis of the up-regulated genes at the various time-points revealed the following dominant pathways: histone modification (1h), angiogenesis, mitosis (3h), G-protein activities (8h), protein synthesis and cell signalling (3d), vascular system development, neuron projection development, neurogenesis and neuron-neuron synaptic transmission (7d), (regulation of) cell differentiation and cell migration (14d). Our results indicate that adult female canary HVC transcriptome is highly sensitive to T. Studying the dynamics of gene expression after T treatment can elucidate the sequence of cellular changes necessary to develop singing behaviour and fully-differentiated songs.

Disclosures: **M. Ko:** None. **C. Frankl-Vilches:** None. **A. Bakker:** None. **M. Gahr:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.04/Y8

Topic: E.01. Neuroendocrine Processes

Support: NIH/NINDS RO1 35467

Title: Intraspecific variation in hormone modulated neuroplasticity in canaries

Authors: *F. N. MADISON[1], G. F. BALL[2,1];
[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Dept. of Psychology, Univ. of Maryland, College Park, MD

Abstract: Previous work in our lab has shown that male canaries of the American singer strain did not exhibit hormone-modulated plasticity in the song control nucleus HVC as can be readily observed in other strains such as the Border canary. In response to high exogenous testosterone (T), male American singers responded in a variable manner. For example, various doses of T did not consistently induce an increase in the volume of the song nucleus HVC. Our data suggests that domestication of the American Singer canary, via human selection perhaps to lock-in a certain level of song quality and quantity, has led to a decrease in neuroplasticity associated with changes in a hormonal action. In order to characterize hormone modulated neuroplasticity in the avian and mammalian brain, the microtubule-associated protein doublecortin (DCX) has been shown to facilitate neuronal migration and final positioning of newly born neurons and is densely expressed in the adult canary HVC thereby serving as a marker of adult neurogenesis. In this study, we investigate possible strain differences in response to T on HVC volume in adult male American singer and Border canaries exposed to identical environmental conditions. Fifteen male American singer (AM) and fifteen male Border canaries (BDR) were housed on a short day 8L:16D light cycle for at least eight weeks, rendering them photosensitive. Birds were then placed into groups of intact (INTACT), castrated (CX), and castrated implanted subcutaneously with one 12mm length Silastic implant filled with crystalline T (CX+T). Immediately after implantation, birds were individually housed in sound attenuated chambers on the 8L:16D light cycle for three weeks. After three weeks of treatment, brains were collected from male AM and BDR canaries, sectioned, and HVC volumes were measured based on Nissl stained sections. Brain sections were also analyzed via immunohistochemistry for DCX and immunopositive cells were counted in HVC. Consistent with our hypothesis, we found that HVC volumes were significantly larger in response to T in BDR than in AM. We also found a significantly higher number of both round and fusiform DCX positive cells in HVC in response to T in BDR than in AM. These data suggest that the process of selection for certain strain phenotypes is implemented by significant changes in hormone-regulated plasticity that reduce behavioral variability in preferred traits.

Disclosures: **F.N. Madison:** None. **G.F. Ball:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.05/Y9

Topic: E.01. Neuroendocrine Processes

Support: NIH/NINDS RO1 35467

Title: Effects of testosterone on microglia in song control nucleus HVC in female canaries

Authors: *G. F. BALL[1,2], F. N. MADISON[2];
[1]Univ. of Maryland, College Park, MD; [2]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Songbirds, such as the canary, demonstrate remarkable seasonal plasticity in the volume of telencephalic brain nuclei that control song behaviors, specifically in the key forebrain nucleus HVC. In the spring, male canary testosterone (T) concentrations increase, stimulating an increase in HVC volume. HVC exhibits a marked sex difference in that female canaries have a smaller HVC volume than males, yet it has been shown that exogenous T in adulthood can stimulate an increase in HVC volume in females. This volumetric increase in HVC induces male-typical songs in females who normally sing songs simpler than males. This hormone-modulated adult seasonal neuroplasticity associated with the increase in circulating T seems to be mediated at least in part by estrogens via the conversion of T to estradiol (E2). E2 is synthesized from T via the enzyme aromatase and is thought to promote the survival and differentiation of new neurons in HVC. Interestingly, mammalian studies have shown that T reduces the occurrence of apoptosis in the sexually dimorphic nucleus of the preoptic area in female and castrated male rats early in development. It has also been shown that microglia are required for the masculinization of brain and behavior during development and that E2 was sufficient in masculinizing the microglia number and morphology in female rats. In this study, we wanted to characterize the distribution of microglia positive cells in HVC in response to exogenous T in adults. Fifteen adult female American singer canaries were surgically implanted with one 12mm length Silastic implant filled with crystalline T for three weeks and then removed (T-REM) for three weeks, implanted with an empty implant (CONT), or implanted with a T-implant for the last three weeks of the study (T-IMP). Immediately after the first implantation, birds were individually housed in sound attenuated chambers on a short day 8L:16D light cycle for six weeks. Brains were collected, fixed, and sectioned for immunohistochemical analysis of the microglial specific marker Iba1. Preliminary results suggest that CONT females had a higher number of Iba1 positive cells in HVC compared to T-IMP females who had fewer immunopositive cells per unit tissue in both HVC and surrounding tissue. T-REM females had fewer Iba1 positive cells in HVC compared to CONT, but had a higher number of Iba1 positive cells bordering HVC. T treatment did result in a change in the morphology of the Iba1 positive cells so that they appear less ramified, which is consistent with them moving toward a more activated state. These data suggest that microglia may be playing a role in hormone-regulated plasticity in songbirds.

Disclosures: G.F. Ball: None. **F.N. Madison:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.06/Y10

Topic: E.01. Neuroendocrine Processes

Support: NIH 5R21DA032747-02

NSF IOS1353263

Title: Female rats liberated for inclusion in neuroscience research

Authors: *J. B. BECKER[1], J. W. LIANG[2], B. J. PRENDERGAST[3];
[1]Univ. Michigan, Ann Arbor, MI; [2]Psychology, Hunter College, City Univ. of New York, New York City, NY; [3]Psychology, Univ. of Chicago, Chicago, IL

Abstract: Not including female rats or mice in neuroscience research has been justified due to the variable nature of female data caused by hormonal fluctuations associated with the female's reproductive cycle. Prendergast et al., (2014 *Neurosci Biobehav Rev* 40:1-5) recently reported in a meta-analysis that female mice are not inherently more variable than male mice across diverse physiological traits. In this study we investigated whether this pattern of variability extends to neuroscience-related traits in rats. PubMed and Web of Science were searched for the period from 8/01/2010 to 7/31/2014 for articles that included (rat AND sex difference) AND (brain OR neuroscience OR neuron). This yielded 562 articles, which were manually reviewed. Only empirical articles using both male and female gonad-intact adult rats, written in English, and including the number of subjects (or a range) were included— resulting in 314 articles for analysis. Methods and data sections were examined and analyses were carried out as described in Prendergast et al. (2014). Data were only used if the mean and standard deviation (SD) or standard error (SEM) could be extracted from the article. Data were extracted from digital image files generated from high-resolution screenshots of article PDFs, and from manuscript tables and text. Vector graphics software was used to quantify the mean and SEM or SD values directly from figure images (in mm), which provided a relative measure of the mean and SD/SEM for each trait. In addition, the results were coded for the type of research being measured (behavior, electrophysiology, histology, neurochemistry, and non-brain measures) and for the strain of rat. Over 6000 data points were extracted for both males and females, and the coefficient of variation (CV; S.D./mean) was calculated for each data point. Data were analyzed by ANOVA followed by two-tailed t-tests. There were no sex differences in trait variability, as indicated by the CV, for any of the measures or overall. The distributions of CV ratios [CV-female/(CV-female+CV-male)] were also analyzed by trait. The CV ratio distribution ranges from 0.0 to 1.0, with CV ratios > 0.5 indicating greater trait-specific CVs in females, and CV ratios < 0.5 indicating greater trait CVs in males. There were significant differences among the CV ratios, driven primarily by the finding that only for the histology measures was the CV ratio > 0.5. For all other measures the CV ratio was < 0.5.

Disclosures: J.B. Becker: None. **J.W. Liang:** None. **B.J. Prendergast:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.07/Y11

Topic: E.01. Neuroendocrine Processes

Support: NSF IOS1353263

Title: Selective activation of estradiol receptors differentially modulates motivation for food in female rats

Authors: *K. YOEST[1], J. A. CUMMINGS[2], J. B. BECKER[1];
[1]Mol. and Behavioral Neurosci. Inst., [2]Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: In females, eating behavior and weight are strongly influenced by levels of circulating gonadal hormones. In normally cycling intact female rodents, ovulation is accompanied by a drop in food intake and overall body weight. Removal of gonadal hormones by ovariectomy results in a dramatic increase in body weight and administration of estradiol to ovariectomized rats results in a significant decrease in meal size and body weight (Asarian and Geary, 2006, Philos Trans R Soc Lond B Biol Sci 361:1251-1263). Recent work has focused on the role of two selective estradiol receptors, ERα and ERβ, in mediating this reduction in feeding behavior. Food intake in ovariectomized rats is decreased by the ERα selective agonist propylpyrazole triol (PPT), but not by the ERβ selective agonist diarylpropionitrile (DPN; Santollo et al, 2007, Am J Physiol Regul Integr Comp Physiol. 293:R2194-201). This study investigates whether the estradiol-mediated decrease in food consumption is accompanied by a decrease in motivation for food. These two aspects of behaviors are dissociable in a number of paradigms and so may be differentially affected by gonadal hormones. Ovariectomized female rats were trained to respond for a food reward on a fixed interval schedule. Females were then hormone primed with either estradiol benzoate (EB), PPT, or DPN for two days, followed by progesterone on the third day in a regimen that induces sexual receptivity (Cummings and Becker, 2012, J Neurosci Methods. 15;204:227-33). Administration of estradiol and progesterone reduced the total number of rewards the animals received as well as the number of responses animals made during each fixed interval. Administration of PPT and progesterone appeared to mimic this effect, while administration of DPN and progesterone resulted in an increase in the total number of rewards the animals received as well as the number of responses per fixed interval. These findings demonstrate that reduced food intake after administration of estradiol is accompanied by a decrease in motivation for food rewards. In addition, activation of ERα appears to be responsible for the effect of estradiol to decrease motivation for food. Interestingly, activation of ERβ had the opposite effect. We found enhanced responding for a palatable food pellet following treatment with DPN. For female rat sexual behavior ERα activates both lordosis and proceptivity, while ERβ modulates this action of ERα (Mazzucco et al, 2008, Behav Brain Res. 5;191:111-7). These results, together with the results reported here, suggest that ERα may play a

role in directing the activation of specific appetitive behaviors which are then modulated up or down by ERβ.

Disclosures: **K. Yoest:** None. **J.A. Cummings:** None. **J.B. Becker:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.08/Y12

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant DA013185

Title: Estrogen-mediated signaling regulates synaptic proteins in the arcuate nucleus of the hypothalamus

Authors: ***A. M. WONG**, L. M. RUDOLPH, P. E. MICEVYCH;
Neurobio., UCLA, Los Angeles, CA

Abstract: Lordosis, a measure of female sexual receptivity in rodents, is regulated by estradiol membrane-initiated signaling (EMS) in the arcuate nucleus of the hypothalamus (ARH). During EMS, membrane estrogen receptor-α (mERα) activates intracellular signaling pathways through the transactivation of metabotropic glutamate receptors (mGluRs). This EMS-initiated regulation of mGluRs promotes the development and maturation of dendritic spines in the ARH, which are required for estradiol-induced lordosis behavior. To better understand the molecular mechanisms of EMS-regulated spinogenesis in the ARH, we examined how estrogen regulated GAP-43 and PSD-95, measures of synaptic sprouting and synapse maturation, respectively. Following a systemic injection of estrogen benzoate (EB; 50 μg), GAP-43 mRNA in the ARH was rapidly induced (40% increase from oil-injected rats 30 min post-EB) and continued to increase until it peaked at 24 h (2.5-fold increase from oil-treated controls). EB-induced transcription of PSD-95 was more gradual, significantly increasing at 24 h post-EB treatment (60% increase from oil controls) with a 2-fold increase in mRNA levels 48 h post-EB, a time point when EB-induced lordosis is maximal. These transcriptional changes in markers of synapse sprouting and maturation are consistent with our previous findings demonstrating that estrogen rapidly induced the formation of immature, filapodial spines in the ARH (< 4 h), but the mature mushroom spines appear later (20-48 h). To support the hypothesis that these EB-induced changes in these synaptic markers are regulated by EMS activation of mGluRs, we injected 100 nmol of LY 367385, a selective mGluR1a antagonist, into the lateral ventricle 30 min prior to systemic EB treatment (50 μg) and measured GAP-43 and PSD-95 in the ARH 24 h later. We found that EB induced GAP-43 mRNA and protein (58% increase) 24 h after EB treatment. Inhibiting mGluR1a with LY367385 suppressed EB induction of GAP-43 (35% decrease) and PSD-95

(40% decrease) mRNAs in the ARH, demonstrating that EB-induced GAP-43 and PSD-95 mRNAs occur via EMS and require mGluRs. Together, these data are consistent with hypothesis that EB-induced synaptic changes required for exhibition of sexual receptivity require rapid spinogenesis in the ARH, and later, maturation of dendritic spines, processes both mediated by mGluR1a-dependent EMS.

Disclosures: A.M. Wong: None. **L.M. Rudolph:** None. **P.E. Micevych:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.09/Y13

Topic: E.01. Neuroendocrine Processes

Support: NIH grant AG041360

Title: Different effects on calcium signaling during and following acute estrogen application in F344 rat basal forebrain neurons and involvement of L-type calcium channels

Authors: *D. A. MURCHISON, D. W. DUBOIS, A. S. FINCHER, W. H. GRIFFITH; Dept. of Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Our lab has been investigating neuronal estrogenic signaling in a rat model of reproductive aging and has identified inhibitory synaptic transmission as an important target of acute estrogenic modulation. We attributed this modulation to the inhibitory effect of 17-β estradiol (17βE) on voltage-gated Na and Ca currents (~35%), as well as on the amplitude of evoked Ca signals (~25%). Here, we elaborate on these findings by using fura-2 microfluorimetry to examine evoked Ca responses before, during and after acute application of 17βE (200 nM) and L-type (CaV1) channel blocker nifedipine (NIF, 10 μM) in acutely dissociated neurons from the basal forebrain (BF) of young adult (2-4 mo) male F344 rats. We found previously that brief (2-10s) focal application of 17βE to BF neurons stimulated small transient Ca signals in a fraction of cells from female rats while being ineffective in males. Also, we observed that the amplitudes of Ca-transients evoked by focal application of elevated potassium solution (Hi K, 20-30 mM) were reduced in the presence of 17βE. We now report that more prolonged applications (3-5 min) of 17βE trigger spontaneous Ca-transients and a persistent elevation in baseline Ca concentration (66 ± 9 nM) in a majority (15/16) of BF neurons from young male rats. We found also that acute 17βE has only a small inhibitory effect on the amplitude of HI K evoked Ca-transients in NIF (12 ± 3 % in 11/20 cells). This result was supported by whole-cell voltage-clamp experiments showing that barium currents through high voltage activated (HVA) Ca-channels in NIF were inhibited by 17βE in 2/7 BF neurons (mean inhibition 15 ± 0.7%). Unexpectedly, after 5-10 minute washout of 17βE and NIF, the amplitude

of Hi K evoked Ca-transients was significantly increased (24 ± 5 %, n=15). We determined that this was partly due to an effect of repetitive stimulation, because neurons (n=12) subjected to repeated Hi K stimulation (13-22 stimuli, 0.3-0.5 s each) over 20 minutes had a 16 ± 44 % increase in Ca-transient amplitude. Tests for a persistent effect of acute 17βE revealed that Ca-transient amplitudes were increased 63 ± 20 % (n=10) over control 5-12 minutes following washout from a 3-5 min application of 17βE without intervening Hi K stimulation. Overall, our data suggest that L-type and other HVA Ca-channels are targets of acute estrogen signaling. Given the role of L-type channels in age-related calcium dysregulation and our earlier results showing that chronic estrogen therapy preserves youthful Ca-buffering properties in BF neurons from ovariectomized reproductively senescent female rats, these findings may help resolve some of the paradox surrounding estrogenic therapy.

Disclosures: D.A. Murchison: None. **D.W. DuBois:** None. **A.S. Fincher:** None. **W.H. Griffith:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.10/Y14

Topic: E.01. Neuroendocrine Processes

Support: FIS/IMSS/PROT/G10/821

UMSNH; Reg. 108907

Title: A Golgi study of the plasticity of dendritic spines in the hypothalamic ventromedial nucleus during the estrous cycle of female rats

Authors: *M. CERVANTES[1], D. A. VELÁZQUEZ-ZAMORA[2], D. GONZÁLEZ-TAPIA[2], I. GONZÁLEZ-BURGOS[3];
[1]Fac. C. Medicas y Biologicas, Morelia, Mexico; [2]Univ. Politécnica de la Zona Metropolitana de Guadalajara, Tlajomulco de Zúñiga, Mexico; [3]Inst. Mexicano del Seguro Social, Guadalajara, Mexico

Abstract: Estradiol-induced plasticity involves changes in dendritic spine density and in the relative proportions of the different dendritic spine types that influence neurons and neural circuits. Such events affect brain structures that control the timing of neuroendocrine and behavioral processes, influencing both reproductive and cognitive functions during the estrous cycle. Accordingly, to investigate the dendritic spine-related plastic changes that may affect the neural processes involved in mating, estradiol-mediated dendritic spine plasticity was studied in type II cells situated in the ventrolateral portion of the ventromedial hypothalamic nucleus (WMN) of female, adult rats. The rats were assigned to 4 different groups (n = 6) in function of

their stage in the estrous cycle: proestrus, estrus, metaestrus, and diestrus. Dendritic spine density and the proportions of the different spine types on type II neurons were analyzed in the ventrolateral region of the VMN of these animals. Dendritic spine density on primary dendrites of VMN type II neurons was significantly lower in metaestrus than in diestrus, proestrus and estrus (with no differences between these latter stages). However, a significant variation in the proportional density of the different spine types was found, with a higher proportion of thin spines in diestrus, proestrus and estrus than in metaestrus. Likewise, a higher proportion of mushroom spines was seen in diestrus and proestrus than in metaestrus, and a higher proportion of stubby spines in estrus than in diestrus and metaestrus. Very few branched spines were found during proestrus and they were not detected during estrus or metaestrus. The different types of dendritic spines in non-projection neurons of the VMN could serve to maintain greater synaptic excitatory activity when receptivity and estradiol levels are maximal. However, they may also fulfill an additional functional role when receptivity and estradiol decline. To date specific roles of the different types of spines in neural hypothalamic activity during the estrous cycle remain unknown and they clearly deserve further study.

Disclosures: M. Cervantes: None. **D.A. Velázquez-Zamora:** None. **D. González-Tapia:** None. **I. González-Burgos:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.11/Y15

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant MH066958

Catherine and Hunter Peirson Endowment

Title: Glucocorticoids desensitize neurons to norepinephrine by enhancing ligand-dependent adrenergic receptor internalization

Authors: *G. L. WEISS, Z. JIANG, J. G. TASKER;
Tulane Univ., New Orleans, LA

Abstract: Glucocorticoids (GC's) are stress related hormones that are released by activation of the hypothalamic-pituitary-adrenal (HPA) axis. GC's regulate peripheral metabolism and feed back on the brain to act on several systems, including the feedback inhibition of the HPA axis. Canonically, GC's mediate transcription by binding to nuclear receptors, a process that can take several hours. Recent findings suggest that GC's have rapid, transcriptionally independent effects, which are likely required for HPA homeostasis. We have previously shown using acute slice electrophysiology that GC's rapidly inhibit the norepinephrine (NE) excitatory drive to

corticotropin releasing hormone (CRH) neurons in the hypothalamus. We hypothesize that CRH neurons are desensitized to NE in response to GC's due to changes in alpha-1 adrenergic receptor (ARα1) trafficking. The GC desensitization of the NE-induced excitation of CRH neurons was reversed when clathrin-mediated endocytosis was blocked, indicating that GC's could be increasing ARα1 internalization. Transfection of N42 immortalized hypothalamic cells with an ARα1b-eGFP construct allowed us to track ARα1b using confocal microscopy. Unexpectedly, GC's had no effect on ARα1b internalization in cultures grown in charcoal-stripped media. However, GC's caused a significant increase in ligand-dependent internalization of the ARα1b receptor in the presence of NE. Additionally, both NE-treated and GC and NE-cotreated cells recovered their membrane ARα1b membrane localization similarly after NE washout, indicating the increase in internalized receptors is not due to a decrease in receptor trafficking back to the membrane. Live-cell imaging revealed increased internalization after only a few minutes, which is consistent with the rapid NE desensitization in our electrophysiological findings. Our data suggest that GC's enhance the NE-induced internalization of ARα1, resulting in complete desensitization of the NE response.

Disclosures: G.L. Weiss: None. **Z. Jiang:** None. **J.G. Tasker:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.12/Y16

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant AG016765

Title: Estrogen and age interact to differentially regulate the hippocampal transcriptome in female rats

Authors: *E. M. WATERS[1], J. D. GRAY[2], W. YIN[3], B. S. MCEWEN[2], A. C. GORE[3]; [1]Sci. Outreach Program, [2]Rockefeller Univ., New York, NY; [3]UT-Austin, Austin, TX

Abstract: Estrogens have both direct and indirect effects on gene transcription via estrogen receptors. In the hippocampus, genomic actions of estradiol modulate plasticity in an estrogen receptor dependent manner. To identify the gene targets by which age and hormone status modify the hippocampus, we conducted RNA sequencing in young (4 month) and middle aged (11 months) female rats that were ovariectomized (OVX) and given estradiol or vehicle capsules for three months. At that time, brains were collected, sectioned and stored in RNALater (same animals as used by Yin et al 2015). The CA1, CA3 and dentate gyrus regions of the hippocampus were individually dissected and RNA extracted. Total RNA from 8-10 individuals was pooled and ribosomal RNA was depleted using the Illumina Ribo-Zero kit to enrich for

messenger RNA, and then preamplified prior to RNA sequencing. Using an Illumina Hi-Seq 2500, 100bp single end reads were collected at a depth of approximately 30M reads per pooled sample. Reads were trimmed and filtered to preserve only high quality reads prior to alignment to the rat genome (Rn5) using TopHat2. Analysis was carried out using Strand (Agilent) to identify differentially expressed genes, novel transcripts and transcript variants. We found that estradiol treatment induced a multitude of transcriptional changes in both young and middle aged OVX females and these effects occurred in an age- and region-specific manner. In both the CA1 and CA3 regions, estradiol treatment resulted in numerous transcriptional events but only 50% of these entities were shared by the young and middle age females. In addition, the results from the CA1 and CA3 were largely unique, suggesting that estradiol's actions in each region is distinct. These results also confirm previous work on synaptic proteins, growth factors, and steroid receptors as well as identified new gene targets related to estrogen-mediated plasticity in the hippocampus. Altogether, these results provide further insights into the ability of hormone replacement to reverse estrogen loss with increasing age.

Disclosures: E.M. Waters: None. **J.D. Gray:** None. **W. Yin:** None. **B.S. McEwen:** None. **A.C. Gore:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.13/Y17

Topic: F.02. Animal Cognition and Behavior

Support: Hungarian Scientific Research Fund Grant OTKA K100722

Title: Estradiol robustly regulates the hippocampal transcriptome in middle-aged, ovariectomized female rats

Authors: *Z. LIPOSITS[1], I. KALLÓ[1], E. HRABOVSZKY[1], N. SOLYMOSI[2], A. RODOLOSSE[3], C. VASTAGH[1], H. AUER[4], M. SÁRVÁRI[1];
[1]Inst. of Exptl. Medicine, Hungarian Acad., Budapest, Hungary; [2]Fac. of Vet. Sci., Szent István Univ., Budapest, Hungary; [3]Functional Genomics Core, Inst. for Res. in Biomedicine, Barcelona, Spain; [4]Functional Genomics Consulting, Palleja, Spain

Abstract: The hippocampus plays indispensable roles in learning and memory. In this area, gonadal hormones including 17β-estradiol (E2) are powerful modulators of neurogenesis, neurotransmission, synaptic plasticity and neuroprotection. In women, menopause is associated with increased risk of memory disturbances which can be attenuated by timely estrogen replacement therapy (ERT). ERT's effects on hippocampus and memory are complex, and depend on responsiveness of the individual, chemical structure and dosage of the estrogen

compound and treatment initiation, among others. In middle-aged rat models of menopause, E2 replacement improves hippocampus-dependent spatial memory (Rodgers SP et al., Endocrinol 151:1194, 2010). In this model, we explored the effect of E2 replacement on hippocampal gene expression. Middle-aged ovariectomized female rats were treated continuously for 29 days with E2 and then, the hippocampal transcriptome was investigated with Affymetrix expression arrays. Microarray data were analyzed by Bioconductor packages and web-based softwares, and verified with quantitative PCR. At standard fold change (FC) selection criterion, 156 genes responded to E2. All alterations but four were transcriptional activation. Prime (FC>10) E2 target genes included transthyretin, klotho, claudin 2, prolactin receptor, ectodin, coagulation factor V, insulin-like growth factor 2, Igfbp2 and sodium/sulfate symporter. Classification of the 156 genes revealed major groups including signaling (35 genes), metabolism (31 genes), extracellular matrix (17 genes) and transcription (16 genes). We selected 33 genes for further studies and all changes were confirmed by real-time PCR. The results suggest that E2 promotes retinoid, growth factor, Otx2 homeoprotein, neurohormone and neurotransmitter signaling, changes metabolism, extracellular matrix composition, transcription, and induce protective mechanisms via genomic effects. We propose that these mechanisms contribute to the beneficial effects of E2 on memory performance. Our findings provide further support for the rationale to develop safe estrogen receptor ligands for the maintenance of cognitive performance in postmenopausal women.

Disclosures: **Z. Liposits:** None. **I. Kalló:** None. **E. Hrabovszky:** None. **N. Solymosi:** None. **A. Rodolosse:** None. **C. Vastagh:** None. **H. Auer:** None. **M. Sárvári:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.14/Y18

Topic: E.01. Neuroendocrine Processes

Support: Faculty of Medicine

Title: An Apiarian Mixture achieves to balance the neuron density in CA3 layer, Serum Estradiol Levels and improves the memory in ovariectomized rats

Authors: *D. A. VÁZQUEZ MATÍAS[1], R. MAYEN DIAZ[2], M. RAMIREZ ESCOTO[3], I. SÁNCHEZ CERVANTES[2], I. LOPEZ MARTINEZ[3], K. PINEDA ROMERO[2], R. GONZALEZ TREJO[2], A. SOLANA ROJAS[2], M. VELAZQUEZ PANIAGUA[2], P. VERGARA ARAGÓN[2]; [2]Dept. of Phisiology Fac. of Med., [3]Cell and Tissue Biol. Dept. Fac. of Med., [1]UNAM, Coyoacán, Mexico

Abstract: The aim for this study was to assess the effect of an Apiarian Mixture on the cognitive impairment and its relation with the CA3 neuron density in an induced menopause rat model.

Methods: 40 Wistar rats 600-800g were divided in four groups of 10 rats each one: a)Control; b)rats with Apiarian Mixture (AM), c) Rats underwent to Ovariectomy (OVX), and d) Ovariectomized rats with Apiarian Mixture (OVX+AM). The AM is made from apiarian derivates such as honey, propolis, royal jelly, pollen and queen-bee larvae. It was administrated vo 0.2 mg/kg/28 days. Short Term Memory (STM) and Long Term Memory (LTM) were evaluated with a passive avoidance test, 10 minutes after the training for STM, and 24 hours for LTM. Blood samples were obtained to determine serum 17β-estradiol levels (SE) by ELISA. Brains were obtained to assess the neuron density (ND) on CA3 layer by optical microscopy. Data were analyzed with One-Way ANOVA and Pearson´s test; p values < 0.001 were considered significant. Results: STM showed a significant decline in the OVX group compared to the other groups. OVX vs control: (280.0±83.0 vs 550.0±46.0), OVX vs AM: (280.0±83.0 vs 600.0±0.0), OVX vs OVX+AM (280.0±83.0 vs 509.9±63.9); the ovariectomized rats who got the AM displayed a recovery in their latency during the test. LTM showed a significant decrease in latency of OVX rats compared to the other groups: OVX vs Control: (190.6±69.3 vs 547.1±78.5), OVX vs AM: (190.6±69.3 vs 600.0±0.0), again, the ovariectomized rats who got the AM displayed a recovery in their latency OVX vs OVX+AM: (190.6±69.3 vs 496.2±61.0). SE showed significant differences, where OVX animals displayed lower SE than the rest of the groups. OVX vs Control: (25.7±9.1 vs 49.03±6.91 pg/ml), OVX vs AM: (25.7±9.1 vs 48.2±6.8 pg/ml) Comparing SE from OVX rats vs OVX+AM, the rats who received the AM improved their SE, similar to SE displayed by control rats: (25.7±9.1 vs 43.98±5.09 pg/ml). In CA3 ND analysis, OXV group displayed a significant increase compared to the other groups: OVX vs Control: (37.8±11.0 vs 22.3±4.6), OVX vs AM: (37.8±11.0 vs 19.9±2.8), while the comparison of OVX vs OVX+AM disclosed a normalization of the neuron density in CA3 in the rats who got the AM: (37.8±11.0 vs 15.1±4.6), showing a ND similar to the control rats. Pearson test, revealed negative association among neuron density and STM r=-0.8508, and LTM r=-0.8591. Conclusion: The components of the AM are able to raise SE in OVX rats up to normal levels, which promote a recovery of the citoprotective function of this hormone. Also we conclude that CA3 layer is sensitive to the elements of the AM, and its neuron density have a strong negative association with STM and LTM.

Disclosures: D.A. Vázquez Matías: None. **R. Mayen Diaz:** None. **M. Ramirez Escoto:** None. **I. Sánchez Cervantes:** None. **I. Lopez Martinez:** None. **K. Pineda Romero:** None. **R. Gonzalez Trejo:** None. **A. Solana Rojas:** None. **M. Velazquez Paniagua:** None. **P. Vergara Aragón:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.15/Y19

Topic: E.01. Neuroendocrine Processes

Support: NIH grant R01MH095248

NIH grant T32MH067564

Title: Sex-specific role of PKA in acute estradiol-induced potentiation of excitatory synaptic transmission in the rat hippocampus

Authors: *A. JAIN, C. S. WOOLLEY;
Northwestern Univ., Evanston, IL

Abstract: 17-β estradiol (E2) is synthesized in the hippocampus and can acutely influence synaptic physiology through non-genomic mechanisms. One acute effect of E2 is to potentiate excitatory synaptic transmission, which occurs in the hippocampus of both males and females. For example, using whole cell voltage clamp recording in rat hippocampal slices, we found that a brief (10 min) bath application of E2 increased the amplitude of synaptically evoked excitatory postsynaptic currents (EPSCs) in ~60% of CA1 pyramidal neurons, by 73±7% in females (n=14) and 80±13% in males (n=13). The remaining E2-nonresponsive cells showed no effect on EPSC amplitude (1±7%, n=10 females, n=8 males). In both sexes, E2-responsive EPSCs remained high after E2 washout. The mechanisms underlying E2-induced EPSC potentiation are unknown, but may involve kinases that act rapidly and are known to regulate other forms of plasticity. Thus, using the same approach, we investigated whether cAMP-activated protein kinase (PKA) contributes to the initiation and/or maintenance of E2-induced EPSC potentiation. PKA activity was inhibited by bath application of H89 (1μM) or membrane-permeant PKI (0.5μM), which have different mechanisms of action. In females, PKA inhibitors completely blocked the E2-induced increase in EPSC amplitude (n=7 H89, n=7 PKI E2-responsive cells). Thus, PKA is required for the initiation of E2-induced EPSC potentiation in females. In contrast, in males, PKA inhibitors failed to block the E2-induced increase in EPSC amplitude (n=9 H89, n=4 PKI). This was true even with a higher dose of H89 (5μM, n=5). Thus, unlike in females, PKA is not required for the initiation of E2-induced EPSC potentiation in males. Further, the magnitude of E2-induced EPSC potentiation with PKA inhibition in males (117±17%) was significantly greater ($p<0.01$, unpaired t-test) compared to that with E2 alone (80±13%), suggesting that, in males, PKA constrains the initiation of E2-induced EPSC potentiation. In contrast to the effects of PKA inhibitors on initiation of E2-induced EPSC potentiation, H89 applied after E2-induced EPSC potentiation was established had no effect in either sex, demonstrating that PKA is not required for maintenance of E2-induced EPSC potentiation (n=7 females, n=6 males). Together, these results demonstrate that although E2 acutely potentiates excitatory synaptic transmission in the hippocampus of both males and females, the molecular mechanisms that underlie this potentiation differ between the sexes. This study adds to a small but growing literature showing sex-specific roles of kinases in neuromodulation, even in brain regions not directly involved in reproduction.

Disclosures: **A. Jain:** None. **C.S. Woolley:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.16/Y20

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant MH095248

Title: Hippocampal excitatory synapses are acutely modulated by estradiol through distinct mechanisms in males and females

Authors: *J. G. OBERLANDER, C. S. WOOLLEY;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: Estradiol (E2) acutely potentiates glutamatergic synaptic transmission in hippocampal CA1 pyramidal cells of both sexes. Separate studies have shown, in males, that E2-induced synaptic potentiation involves an estrogen receptor (ER)β-dependent increase in postsynaptic sensitivity to glutamate (Kramar et al., 2009) and, in females, that E2-induced synaptic potentiation involves an ERβ-dependent increase in presynaptic glutamate release probability (Smejkalova & Woolley, 2010). Whether these distinctions reflect sex differences or other factors has been unclear. In preliminary work, we used whole cell recording and two-photon (2p) uncaging of glutamate at individual dendritic spines to investigate pre- and postsynaptic effects of E2. We found that E2 increases miniature EPSC (mEPSC) frequency, mEPSC amplitude, and the amplitude of 2p glutamate uncaging evoked EPSCs (2pEPSCs) in both males and females, confirming pre- and postsynaptic effects of E2 in each sex. Consistent with other studies, E2 regulation of mEPSCs occurred in a subset of cells (55-60%). Interestingly, pre- and postsynaptic effects often occurred in separate cells suggesting distinct mechanisms. Here, we used the same approaches combined with ER-selective agonists to investigate which ERs mediate pre- and postsynaptic effects of E2, and we compared males and females. Agonists selective for ERα (PPT,100nM), ERβ (WAY200070,10nM), or GPER1(G1,100nM) were applied to hippocampal slices from adult female or male rats for 10 min, followed by E2 for 10 min. In females, WAY increased mEPSC frequency in 6 of 13 (46%) cells, with no effect on mEPSC or 2pEPSC amplitude. In males, WAY had no effect on mEPSC frequency, but increased mEPSC amplitude in 4 of 16 cells (25%) and increased 2pEPSC amplitude at 16 of 51 (31%) dendritic spines. PPT had no effect on mEPSCs or 2pEPSCs in females (n=10 cells, 33 spines), whereas in males, PPT increased mEPSC frequency in 9 of 16 (56%) cells, with no effect on mEPSC amplitude or the amplitude of 2pEPSCs (n = 54 spines). In females, G1 increased mEPSC amplitude in 7 of 18 (39%) cells with no effect on mEPSC frequency, and increased 2pEPSC amplitude at 13 of 52 (25%) dendritic spines. In males, G1 had no effect on mEPSCs (n=22) or 2pEPSCs (n= 67). Together, these results show that, in females, E2 increases glutamate release probability through ERβ and glutamate sensitivity through GPER1. In males, E2 increases glutamate sensitivity through ERβ and glutamate release probability through ERα.

Thus E2 acutely potentiates glutamatergic synapses in the hippocampus of both males and females, but works through different ER-dependent mechanisms in each sex.

Disclosures: J.G. Oberlander: None. **C.S. Woolley:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.17/Y21

Topic: E.01. Neuroendocrine Processes

Title: Progranulin deficiency attenuates estrogen-induced increase of adult neurogenesis in the hippocampus

Authors: *M. DOKE, T. MATSUWAKI, K. YAMANOUCHI, M. NISHIHARA;
Vet. Physiology, the Univ. of Tokyo, Tokyo, Japan

Abstract: Neurogenesis in the adult mammalian brain is known to occur in predominantly two regions, the subgranular zone (SGZ) of the dentate gyrus and the subventricular zone of the lateral ventricle. It is generally accepted that estrogen has a function to protect neurons by promoting neurogenesis as well as attenuating neuroinflammation and neurodegeneration. Estrogen also has a function to induce sex differences of the brain during the perinatal period in rodents, and we have suggested that progranulin (PGRN), a multifunctional growth factor, mediates this function of estrogen. In addition, we have recently demonstrated that PGRN is involved in voluntary exercise-induced neurogenesis and suppression of neuroinflammation after traumatic brain injury. Thus, in the present study, we hypothesized that the neuroprotective functions of estrogen might be also mediated by PGRN, focusing on the adult neurogenesis in the SGZ of the hippocampus. In all the experiments, female C57BL/6J wild-type (WT) mice or PGRN-deficient (KO) mice of the same background were used 1 week after ovariectomy. First, we investigated neurogenesis in WT mice 4 and 24 hours after injection of estradiol benzoate or 8 days after implanting silastic tubing containing 17β-estradiol. The brains were dissected out 2 hours after the injection of bromodeoxyuridine (BrdU), a thymidine analogue, and immunohistochemically analyzed. Estrogen increased the number of the BrdU-immunoreactive (ir) cells 4 hours after treatment, but thereafter no stimulatory effect of estrogen on neurogenesis was discernible. Subsequently, we compared the neurogenesis between WT and KO mice 4 hours after injection of estrogen. The estrogen-induced increase in the number of BrdU-ir cells seen in WT mice was not observed in KO mice. We also performed qPCR to evaluate gene expression of PGRN and other growth factors known to be related to neurogenesis, i.e., NGF, BDNF, IGF-1 and VEGFB, in the hippocampus 4 hours after estrogen injection. The results showed that neither genotypes nor estrogen treatment affected gene expression of these growth factors, implying the involvement of these growth factors in cooperative neuroprotective action

of estrogen and PGRN is unlikely. In summary, it is suggested that estrogen only acutely enhances hippocampal neurogenesis and that PGRN is prerequisite for this function of estrogen.

Disclosures: **M. Doke:** None. **T. Matsuwaki:** None. **K. Yamanouchi:** None. **M. Nishihara:** None.

Poster

344. Steroids and Plasticity

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 344.18/Y22

Topic: E.01. Neuroendocrine Processes

Support: The Senior Research Fellowship award (45/13/2011-BMS/Immunology) to P. Kumar from Indian Council of Medical Research (ICMR) is duly acknowledged.)

Title: Role of estrogen and tamoxifen in regulation of immune system and neuroprotection in female rat hippocampus

Authors: ***P. KUMAR**[1], P. DHAR[1], R. MEHRA[2];
[1]AIIMS, New Delhi, India; [2]Anat., HIMSR, New Delhi, India

Abstract: Background: Ever since the first demonstration of estrogen concentrating cells in the brain by Donald Pfaff in early 1960s, the role of this female sex hormone has been under immense scanning in different areas of brain. The effects of estrogen in the central nervous system extend far beyond its predominant role in reproduction. The two most widely acclaimed non-reproductive effects of estrogen are related to i) memory and cognition and ii) neuroprotection. Postmenopausal aging is accompanied with decline in estrogen levels in females, which predispose them to a multitude of age related pathophysiological events including the cognitive decline and Alzheimer's disease. A decent amount of evidence associates the estrogen (E2, 17β estradiol) with hippocampal and cerebellar activity. Presence of estrogen receptors (ERs) in the learning and memory related brain areas gives further evidence of them being a few of the target brain regions for the hormone activity. Existence of ERs in other brain areas further expands the view of pleotropic nature of this hormone. In our current research we have further explored the role which estrogen may have in crosstalk between the nervous, endocrine and immune systems in female brain regions like hippocampus in view of the reports of heightened inflammatory events in neurodegeneration. **Methods**: Ovariectomized rat model was used in the present investigations. Immunohistochemistry, western blot, qRT-PCR and ELISA techniques were used for proteomic and genomic analyses. Our findings have revealed that ovariectomy (OVX, hormone depletion) leads to decreased synaptic activity, degenerative cytoarchitectural changes, microglial activation, altered levels of ERs, anti-apoptotic and other key signalling proteins and concomitant changes in the expression of complement proteins (C1q,

C3 and C3aR) and pro- and anti-inflammatory cytokines (IL-6, TNF-α and IL-10) in these brain areas. We have further observed that long-term estrogen therapy to OVX rats maintains synaptic plasticity (synaptophysin) and regulates microglial activity, apoptotic molecules (Bax and Bcl2), complement proteins and pro-inflammatory cytokines. **Conclusion**: These findings put forth a new perspective on the neuroprotective and neuromodulatory effects of estrogen, which may also include anti-inflammatory response via regulating the neuro-immune response and levels of complement system and pro-inflammatory cytokines together with anti-apoptotic protein regulation. **Ethical Statement**: This study was performed in strict accordance and duly approved by the institutional animal ethics committee.

Disclosures: P. Kumar: None. **P. Dhar:** None. **R. Mehra:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.01/Y23

Topic: E.02. Neuroimmunology

Support: NIAAA Grant

Title: Ethanol modulates CNS and systemic immune responses through HMGB1, IL-1β and HMGB1-containing microparticles

Authors: *L. G. COLEMAN, JR[1], J. ZHOU[2], C. LONERGAN[2], F. CREWS[3];
[1]Univ. of NC- Chapel Hill, Durham, NC; [2]UNC-Chapel Hill, Chapel Hill, NC; [3]UNC-Chapel Hill, Chapel HIll, NC

Abstract: Alcohol modulates innate immune signaling in the brain and periphery through the release of multiple signaling molecules, including the danger associated molecular pattern (DAMP) molecule HMGB1. Expression of brain HMGB1 protein has been found to be elevated in human alcoholics and correlates with lifetime drinking level and age of onset of drinking. Acutely, alcohol increases gut permeability leading to endotoxin released into the blood and DAMP receptor activation. We investigated the CNS and systemic effects of alcohol on innate immune activation. In mice, ethanol (6mg/kg, i.g.) caused a rapid increase in HMGB1 in the plasma that was sustained for 24 hours (2-fold maximum increase, $p<0.05$) and returned to control levels by 48 hours post-treatment. This was associated with an acute increase in plasma IL-1β, which peaked at 6 hours (20% increase, $p<0.002$). HMGB1 protein expression in the brain followed a similar expression pattern, peaking at 1 hour (96% increase, $p<0.05$). Brain expression on inflammasome component caspase-1 also peaked at 1h (62%, $p<0.007$) followed by a transient reduction in expression beyond 6 hours. BV2 microglia treated with of ethanol (0.5%) secreted HMGB1 into the media within 6 hours (37% increase, $p<0.002$) without any

increase in toxicity as measured by LDH. This secretion was enhanced by the addition of the autophagy promoting mTORC1 inhibitor rapamycin (84% increase above control, $p<0.0001$) and reduced by the autophagasome inhibitor Wortmannin to control levels indicating a role of autophagy machinery in secretion of HMGB1 by microglia. SH-SY5Y neurons showed no changes in intracellular or secreted HMGB1 at 6h. However, SH-SY5Y intracellular HMGB1 had increased by 12 hours post-ethanol washout (57%, $p<0.05$). Using a neonatal hippocampal slice culture model to assess a system containing both neurons and glia, we found that 96h ethanol treatment caused a dose dependent increase in cellular (EC50 67.1mM, maximum increase 73.8%) and released HMGB1 (media and microparticle fraction). Released HMGB1 expression had a maximum increase of 17% (EC50 37.2mM) in the media microparticle (MP) fraction and 27% (EC50 27.9) in MP-depleted media. Flow cytometric analysis of media MP revealed a 32% increase in HMGB1 + MP of neuronal origin ($p<0.05$) in response to ethanol (25-100mM). Thus, ethanol causes differential transient microglial and neuronal release of HMGB1 in response to ethanol. Further, ethanol causes the release of neuron-derived HMGB1+ microparticles that may participate in the systemic inflammatory response to ethanol.

Disclosures: **L.G. Coleman:** None. **J. Zhou:** None. **C. Lonergan:** None. **F. Crews:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.02/Y24

Topic: E.02. Neuroimmunology

Support: CONACYT 220598

DGAPA IG 200314

PAEP

Title: Activation of the suprachiasmatic nucleus following salmonella infection

Authors: *R. MÉNDEZ-HERNÁNDEZ, G. SUÁREZ-PÉREZ, R. M. BUIJS;
Inst. De Investigaciones Biomédicas, UNAM., México, Mexico

Abstract: The suprachiasmatic nucleus (SCN) is involved in maintaining circadian physiology. Recently, it has been suggested that it participates importantly in modulating the immune response to bacterial lipopolysaccharide. Moreover it has been shown that the degree of Salmonella infection depends on the time of inoculation, suggesting that as a master clock, the SCN might be also involved. In this study we evaluated the SCN response to Salmonella infection. Wistar rats were inoculated with Salmonella at two different time points (day and night) and brain activation 72 hours after inoculation was assessed by c-fos

inmunohistochemistry. The time of inoculation was shown to have an influence in the outcome of the infection. Activity in several brain regions including the SCN was changed in response to Salmonella in a time point-dependent manner. These findings support a role for the SCN in the outcome of the salmonella infection.

Disclosures: R. Méndez-Hernández: None. **G. Suárez-Pérez:** None. **R.M. Buijs:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.03/Y25

Topic: E.02. Neuroimmunology

Support: Universidad de Guadalajara 222769 PRO-SNI 2014

CONACyT-México 2015-305861

Title: Interleukin-17A varies according to treatment, age, and time of disease evolution in patients with relapsing-remitting multiple sclerosis

Authors: J. J. GUERRERO-GARCÍA[1], V. A. CASTAÑEDA-MORENO[1], N. TORRES-CARRILLO[1], J. F. MUÑOZ-VALLE[1], O. K. BITZER-QUINTERO[3], D. M. PONCE-REGALADO[1], M. A. MIRELES-RAMÍREZ[4], Y. VALLE-DELGADILLO[1], *D. ORTUNO[2]; [1]Inst. de Investigación en Ciencias Biomédicas, [2]Univ. de Guadalajara, Guadalajara, Mexico; [3]Ctr. de Investigación Biomédica de Occidente, [4]Hosp. de Especialidades CMNO, Inst. Mexicano del Seguro Social, Guadalajara, Mexico

Abstract: Multiple sclerosis (MS) is an immune-mediated demyelinating disease that affects the central nervous system. Relapsing-remitting (RRMS) is the most frequent form of MS and its defining feature comprises fluctuating disease activity, in which the patient undergoes clinical relapses separated by periods of clinical stability. The immune system plays an important role in axonal demyelination, involving T-cell subtypes such as Th1, Treg, and Th17, which characteristically produce Interferon gamma (IFN-γ), Interleukin-10 (IL-10), and Interleukin-17 (IL-17A), respectively. Glatiramer acetate (GA) and Interferon beta (IFN-β) are the first-line treatment for RRMS approved by U.S. Federal Drug Administration (FDA). The objective of this study was to explore for the possible existence of different subgroups of patients with MS according to treatment, gender, age, and disease evolution time. In the present work, we determined serum levels of IFN-γ, IL-10, and IL-17A in 82 samples of RRMS (50 females and 32 males) treated with GA and IFN-β. We stratified patients by treatment, gender, age, and disease evolution time. Subsequently we correlated these independent variables with the concentrations of the previously mentioned cytokines. Results showed that treatment exerted possible differential effects depending on disease evolution time and evidence of the existence of

different subgroups of patients with MS as follows: i) male or female under or over the age of 40 years; ii) disease duration according to treatment; and iii) classification according to fluctuating levels of IFN-γ, IL-10, and IL-17A in the following three stages of disease evolution: <5 years; between 5 and 10 years, and after 10 years. These subgroups must be taken into account for the clinical follow-up of patients with MS in order to provide them with a more personalized treatment, and also for a detailed analysis of the disease's progress, in an attempt to comprehend fluctuations and clinical variability by means of better understanding of the disease's intrinsically physiological variables.

Disclosures: J.J. Guerrero-García: None. **V.A. Castañeda-Moreno:** None. **N. Torres-Carrillo:** None. **J.F. Muñoz-Valle:** None. **O.K. Bitzer-Quintero:** None. **D.M. Ponce-Regalado:** None. **M.A. Mireles-Ramírez:** None. **Y. Valle-Delgadillo:** None. **D. Ortuno:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.04/Y26

Topic: E.02. Neuroimmunology

Support: SHRF EG

NSERC DG

Title: Reelin expression in endothelial cells: an electron microscopy study

Authors: *H. J. CARUNCHO[1], E. Y. FENTON[1], R. ROMAY-TALLON[1], L. E. KALYNCHUK[2], E. PEREZ-COSTAS[3];
[1]Col. of Pharm. and Nutr., [2]Col. of Med., Univ. of Saskatchewan, Saskatoon, SK, Canada; [3]Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Background*:* Reelin expression and function have been extensively studied in the brain, although its expression has been also reported in other tissues including blood. This raises the possibility that reelin might be able to cross the blood-brain barrier, which could be functionally relevant. Up-to-date no studies have been conducted to assess if reelin is present in the blood-brain barrier, which is mainly constituted by tightly packed endothelial cells. In this report we assessed the expression of reelin in brain capillaries using immunocytochemistry and electron microscopy. **Results**: At the light microscope, reelin immunolabeling appeared in specific endothelial cells in brain areas that presented abundant diffuse labeling for this protein (e.g., layer I of the cortex, or the *stratum lacunosum moleculare* of the hippocampus), while it was mostly absent from capillaries in other brain areas (e.g., deeper cortical layers, or the CA1 layer of the hippocampus). As expected, at the electron microscope reelin labeling was observed in neurons of the cortex, where most of the labeling was associated with the rough endoplasmic

reticulum. Importantly, reelin was also observed in some endothelial cells located in small capillaries, which confirmed the findings obtained at the light microscope. In these cells, reelin labeling was located primarily in caveolae (i.e., vesicles of transcytosis), and associated with the plasma membrane of the luminal side of endothelial cells. In addition, some scarce labeling was observed in the nuclear membrane. **Conclusions:** The presence of reelin immunolabeling in brain endothelial cells, and particularly in caveolar vesicles within these cells, suggests that reelin and/or reelin peptides may be able to cross the blood-brain barrier, which could have important physiological, pathological, and therapeutic implications.

Disclosures: H.J. Caruncho: None. **E.Y. Fenton:** None. **R. Romay-Tallon:** None. **L.E. Kalynchuk:** None. **E. Perez-Costas:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.05/Y27

Topic: E.02. Neuroimmunology

Support: NIH-NIDCR #DE021888 (OJI)

Title: Role of toll-like receptor 4 in mediating oxidative stress-induced proinflammatory state in macrophages

Authors: *Y. ZHANG, O. J. IGWE;
Univ. of Missouri-Kansas City, Kansas City, MO

Abstract: **Background:** Disturbances in reduction-oxidation (redox) equilibrium of tissue can lead to inflammatory state, which is a mediating factor in many human diseases. However, the mechanism (s) by which oxidative stress can activate an inflammatory response have not been fully elucidated**.** Emerging evidence suggest that Toll-like receptor 4 (TLR4) activation is involved in mediating this response. **Objective:** This study will determine the role of TLR4 in mediating oxidative stress-induced proinflammatory state in macrophages **Methods:** SIN-1 and potassium peroxychromate (PPC) were used as prooxidant sources and a TLR4-specific agonist LPS-EK was used as the positive control. CLI-095, was used to block signal transduction following TLR4 activation. We used murine macrophage RAW-Blue cells chromosomally integrated with secreted embryonic alkaline phosphatase (SEAP) inducible by nuclear factor-κB (NF-κB). Intracellular reactive oxygen species (iROS) production was visualized and quantified by fluorescence imaging and flow cytometry, respectively. Lipid peroxidation were quantified by malondialdehyde (MDA) assay and 4-hydroxynonenal (4-HNE) ELISA. Total antioxidant capacity within cells was quantified by antioxidant assay kit. Activation of NF-κB was quantified by measuring DNA binding activity in the nuclear fraction by TransAM assay, and by measuring

the levels of SEAP release with QUANTI-Blue assay. Expression and phosphorylation of inhibitory κBα (IκBα) were determined by Western blot. Tumor necrosis factor α (TNFα) and Interleukin 10 (IL-10) released into the media were measured by ELISA. **Results:** We found that pro-oxidants treatment increased iROS production and lipid preoxidation, but decreased total antioxidant capacity. Consistent with TLR4 agonist LPS-EK, prooxidants evoked increased level of transcriptionally active form of NF-κB p65 and subsequent SEAP released, which were blocked by pretreatment with CLI-095. In addition, prooxidants treatment decreased protein expression of IκBα with enhanced phosphorylation at Tyr42. Finally, prooxidants and LPS-EK enhanced TNFα production but did not change IL-10 production causing imbalance between cytokines, which can be inhibited by CLI-095. **Conclusion:** Taken together, the results indicate that TLR4 mediated prooxidants induced inflammation response by inducing NF-κB activation in macrophages. Therefore, oxidant stress can serve as an initiator of inflammatory processes that maintain many chronic diseases.

Disclosures: Y. Zhang: None. **O.J. Igwe:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.06/Y28

Topic: E.02. Neuroimmunology

Title: Microglia regulate cell proliferation in the neonatal hippocampus in a sex-dependent manner

Authors: *L. H. NELSON[1,2,3], A. GALAN[4], K. M. LENZ[4,3,2];
[2]Group in Behavioral Neuroendocrinology, [3]Dept. of Neurosci., [4]Dept. of Psychology, [1]Ohio State Univ., Columbus, OH

Abstract: Several neuropsychiatric disorders have been shown to involve altered immune signaling during development and also show a significant sex bias with prevalence toward males. We and others have shown that several regions of the male brain have significantly greater numbers of activated microglia, the innate immune cell in the CNS, during development than the female brain (Lenz et al., 2013, J Neurosci; Schwarz et al., 2012 J Neurochem). We hypothesize that microglia may contribute to sex differences in both normal and abnormal brain development. Microglia regulate the number of proliferating cells in the developing cortex by releasing diffusible factors and phagocytizing healthy progenitors (Cunningham et al., 2013, J Neurosci; Shigemoto-Mogami et al., 2014, J Neurosci). During development there is a basal sex difference in hippocampal neurogenesis in rats, with males having more neurogenesis than females (Zang et al., 2008, Eur J Neurosci). In these studies, we sought to determine whether microglia regulate the sex-specific rate of proliferation in the neonatal hippocampus. Rats were treated by bilateral

intracerebroventricular injection of minocycline or clodronate on postnatal days (PN) 1-2. Minocycline inhibits activated microglia, and clodronate ablates microglia. BrdU was injected on PN2 to label actively dividing cells. Brains were collected on PN3 for immunohistochemical analysis. Minocycline decreased proliferation in the dentate gyrus (DG) of males, but not females, as determined by the number of BrdU+ cells. Similarly, clodronate decreased proliferation in males to female-typical levels. Ongoing studies will determine the effect of clodronate in females. These results suggest that microglia support sex-specific rates of proliferation in the DG. We also analyzed microglial morphology by counting and categorizing Iba-1 positive cells in the DG. We found that females had more phagocytic microglia than males. There was a positive correlation between the number of amoeboid microglia and BrdU+ cells across groups, but no correlation between the number of phagocytic microglia and BrdU+ cells, suggesting that amoeboid microglia may be responsible for sex differences in proliferation, and that phagocytic microglia may be responsible for another sex-specific process in the hippocampus. Future studies will determine what cells are being phagocytized by microglia, and what microglial-factors may regulate proliferation. Examining the role of microglia in the developing brain is important for understanding how they contribute to the development of neuropsychiatric disorders following perinatal perturbations, such as stress or infection.

Disclosures: **L.H. Nelson:** None. **A. Galan:** None. **K.M. Lenz:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.07/Y29

Topic: E.02. Neuroimmunology

Support: German Research Foundation (SFB TRR 43)

Title: Actin dynamics shape microglia effector functions

Authors: ***R. UHLEMANN**[1], K. GERTZ[2], W. BOEHMERLE[2], T. SCHWARZ[2], C. NOLTE[3], D. FREYER[2], H. KETTENMANN[3], M. ENDRES[2], G. KRONENBERG[2];
[1]Exptl. Neurol., [2]Charité, Berlin, Germany; [3]Max Delbrueck Ctr. for Mol. Med., Berlin-Buch, Germany

Abstract: Impaired actin filament dynamics have been associated with cellular senescence. Microglia, the resident immune cells of the brain, are emerging as a central pathophysiological player in neurodegeneration. Microglia activation, which ranges on a continuum between classical and alternative, may be of critical importance to brain disease. Using genetic and pharmacological manipulations, we studied the effects of alterations in actin dynamics on microglia effector functions. Disruption of actin dynamics did not affect transcription of genes

involved in the LPS-triggered classical inflammatory response. By contrast, in consequence of impaired nuclear translocation of phospho-STAT6, genes involved in IL-4 induced alternative activation were strongly downregulated. Functionally, impaired actin dynamics resulted in reduced NO secretion and reduced release of TNF and IL-6 from LPS-stimulated microglia and of IGF-1 from IL-4 stimulated microglia. However, pathological stabilization of the actin cytoskeleton increased LPS-induced release of IL-1beta and IL-18, which belong to an unconventional secretory pathway. Reduced NO release was associated with decreased cytoplasmic iNOS protein expression and decreased intracellular arginine uptake. Furthermore, disruption of actin dynamics resulted in reduced microglia migration, proliferation and phagocytosis. Finally, baseline and ATP-induced [Ca2+]int levels were significantly increased in microglia lacking gelsolin, a key actin-severing protein. Together, the dynamic state of the actin cytoskeleton profoundly and distinctly affects microglia behaviors. Disruption of actin dynamics attenuates M2 polarization by inhibiting transcription of alternative activation genes. In classical activation, the role of actin remodeling is complex, does not relate to gene transcription, and shows a major divergence between cytokines following conventional and unconventional secretion.

Disclosures: **R. Uhlemann:** None. **K. Gertz:** None. **W. Boehmerle:** None. **T. Schwarz:** None. **C. Nolte:** None. **D. Freyer:** None. **H. Kettenmann:** None. **M. Endres:** None. **G. Kronenberg:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.08/Y30

Topic: E.02. Neuroimmunology

Title: Contraction regulates CCL5 secretion in C2C12 myotubes -Potential roles of myokines on peripheral nervous system-

Authors: *H. SATO, T. SHIBAGAKI, K. SATO, T. NEDACHI;
Life Sci., Toyo Univ., Gunma, Japan

Abstract: Microenvironment of peripheral nervous systems (PNS) that are regulated by neuron and glia surrounding PNS is not only important for maintaining normal neuronal activities, but also for protecting neurons from various deleterious stresses. Many researches focused on the neuron-glia and/or glia-glia interaction and several soluble factors secreted by these cells have been implicated in nervous system morphogenesis. However, the microenvironment of PNS could be not only regulated by neuron and glia, but also by the other cell types. Recently, secreted factors from skeletal muscle, also referred to as myokines, were identified and recognized as important mediators of physical exercise. In this study, by utilizing C2C12

contractile model, we attempted to identify novel myokines that potentially modified the microenvironment of PNS. Differentiated C2C12 myotubes were subjected to electrical pulse stimulation (EPS) to induce contraction, thereby analyzed secreted proteins by membrane antibody arrays (Proteome Profiler). Several novel myokines were successfully identified by this method, particularly, we found two myokines, including Chemokine (C-C motif) ligand 5 (CCL5), were significantly reduced by exercise. Quantitative PCR analysis revealed that the exercise-dependent reduction of CCL5 was regulated by gene expressional control. In addition, we found exercise-dependent AMP kinase activation was sufficient for reducing secreted CCL5 levels. Moreover, the inhibition of phosphatidylinositol 3-kinase activity by LY294002 diminished this exercise-dependent CCL5 reduction. In conclusion, we identified CCL5 as a novel myokine that secretion is reduced by skeletal cell contraction, and this reduction is mediated by AMP kinase pathway. It has been demonstrated that CCL5 is secreted from neurons following peripheral nerve injury and appears to be important factor for neuroprotection. Thus, exercise may modify microenvironment of PNS via regulating myokine production, thereby influence on these neuronal protective process.

Disclosures: **H. Sato:** None. **T. Shibagaki:** None. **K. Sato:** None. **T. Nedachi:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.09/Y31

Topic: E.02. Neuroimmunology

Support: VA Merit award to RB

T32 MH016259-35

Title: NLRP3 inflammasome activity demonstrates a diurnal pattern within the somatosensory cortex

Authors: ***M. R. ZIELINSKI**[1], D. GERASHCHENKO[2], S. A. KARPOVA[2], R. W. MCCARLEY[3], R. BASHEER[2];
[1]Harvard Univ. and Veterans Affairs Boston Healthcare Syst., West Roxbury, MA; [2]Harvard Med. Sch. and VA Boston Healthcare Syst., West Roxbury, MA; [3]Harvard Med. Sch. and VA Boston Healthcare Syst., Brockton, MA

Abstract: Inflammasomes are oligomeric protein complex assemblies found in cells throughout the body including neurons, astrocytes, and microglia. The nucleotide-binding domain of leucine-rich family pyrin-containing 3 (NLRP3) component of the inflammasome recognizes pathogen-associated molecular patterns (PAMPs), such as extracellular adenosine tri-phosphate (ATP). In turn, the assembly of a carboxyl-terminal caspase-recruitment domain (ASC)

component of the inflammasome is recruited into the complex. The recruitment of ASC is required for inflammasome activation. The NLRP3 inflammasome activates caspase-1, which converts the pro-form of interleukin-1 beta (IL-1β) into its mature active form. IL-1β is well-documented to be enhanced in the cortex during times of the day when sleep propensity is high. We examined the diurnal molecular activation of the NLRP3 inflammasome within the somatosensory cortex. Male NLRP3 knockout (KO) mice and C57BL/6 wild-type controls were sacrificed at the beginning of light onset [zeitgeber (ZT) 0; a time of high sleep propensity following the active dark period] and at the beginning of dark onset (ZT 12). Somatosensory cortex tissue was homogenized, RNA was extracted, and cDNA was made. The gene expression analysis of NLRP3, ASC, and IL1β mRNA levels was performed using real-time polymerase chain reaction analysis. Somatosensory cortex tissue was also homogenized and processed for caspase-1 activity using a kinetic enzyme-linked immunosorbent assay (ELISA) and the protein levels of IL-1β by ELISA. WT mice exhibited significant enhancements in NLRP3, ASC, and IL-1β mRNA levels, caspase-1 activity, and IL-1β protein levels during the beginning of the light period (ZT 0) compared to the beginning of the dark period (ZT 12). In contrast, NLRP3 KO mice showed no significant differences in NLRP3, ASC, or IL-1β mRNA levels, caspase-1 activity, or IL-1β protein levels between light (ZT 0) and dark periods (ZT 12). These data indicate that diurnal fluctuations in inflammasome activation occur within the somatosensory cortex that coincide with times of sleep propensity, which suggests that the NLRP3 inflammasome might be involved in sleep regulation, perhaps by enhanced ATP production induced by waking activity.

Disclosures: **M.R. Zielinski:** None. **D. Gerashchenko:** None. **S.A. Karpova:** None. **R.W. McCarley:** None. **R. Basheer:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.10/Y32

Topic: E.02. Neuroimmunology

Support: E.R.C Grant 232835

E.R.C Grant 309788

EU FP7 Grant 279017

Title: IFN-beta is a master regulator of microglial phenotype in aging

Authors: *A. DECZKOWSKA[1], O. MATCOVITCH[1], K. BARUCH[1], I. AMIT[2], M. SCHWARTZ[1];
[1]Neurobio., [2]Immunol., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Microglial activity is essential for brain maintenance and repair. Therefore, aging-related changes in microglial phenotype are viewed as a factor contributing to brain function decline in aging. Recently we found, in aged mice and human samples, that the choroid plexus (CP), an epithelial tissue that forms blood-cerebrospinal fluid-barrier (BCSFB), expresses type I interferon (IFN-I) response program, including IFN-β, a pleiotropic cytokine, which, depending on the context, can display both anti- and pro- inflammatory properties. Here, we hypothesized that CP-derived IFN-β is secreted to the CSF and contributes to the age-related changes in microglia, which hold a potential to robustly respond to IFNs-I. To challenge this hypothesis, we performed high throughput RNA-Sequencing of microglia of aged mice following transient blockage of IFN-I receptor (IFNAR) within the brain's territory. In these settings we found that IFNAR inhibition contributed to broad changes in aged microglia expression profile. Specifically, we found, that clusters of genes related to "antigen presentation" (e.g. B2M, CD74) and "defense response" (e.g Itgax, Oasl1 2'-5', Isg15), the expression of which was upregulated in aging, was down-regulated following IFNAR blockage. Conversely, a gene cluster related to "cognition", "learning and memory" and "behavior", the expression of which was down-regulated in aging, was up-regulated in aged mice microglia 7 days after IFNAR blockage, a time-point when the treated mice showed elevated cognitive ability. These results suggest that IFN-β is involved in shaping aged microglia pro-inflammatory phenotype, with possible consequences for cognitive decline and reduced brain plasticity.

Disclosures: A. Deczkowska: None. **O. Matcovitch:** None. **K. Baruch:** None. **I. Amit:** None. **M. Schwartz:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.11/Y33

Topic: E.02. Neuroimmunology

Title: Sex differences in the microglial responses to cerebral ischemia

Authors: *M. M. GAUDIER-DIAZ, N. ZHANG, A. DEVRIES;
Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Cerebral ischemia, caused by cardiac arrest and stroke, is a leading cause of death and disability worldwide. Thus, it is important to find ways to prevent it, facilitate recovery, and improve the quality of life of survivors. Previous research demonstrates that social isolation increases neuroinflammation, neuronal degeneration, and functional impairment following cerebral ischemia. We hypothesize that microglia, the innate immune cells of the central nervous system, modulate the detrimental effects of social isolation on ischemic outcomes. Environmental stressors can sensitize microglia to respond in an exaggerated manner upon

further immune stimulation; by triggering similar mechanisms, it is plausible that the psychological stressor of social isolation can also sensitize microglia. To study the effect of social environment on the microglial response to cerebral ischemia, mice were pair housed (n=24) or socially isolated (n=24), and then a week later exposed to cardiac arrest/cardiopulmonary resuscitation (CA/CPR; n=12/group) or the sham procedure (n=12/group). Samples were collected 24 h later. The brain was dissected, minced, homogenized and resuspended in a percoll gradient to provide an enriched sample of microglia. Then, gene expression of pro-inflamamtory (IL-1β, IL-6, TNFα) and anti-inflammatory (IL-10) cytokines was assessed. Among individually housed male mice that received the CA/CPR procedure, there was increased microglial gene expression of IL-1β and IL-6 relative to sham controls; in contrast, gene expression among pair housed males did not differ between CA/CPR and sham groups. This suggests that social isolation exacerbates ischemia-induced neuroinflammation through microglial production of pro-inflammatory cytokines. In contrast, among female mice there was increased microglial gene expression of pro-inflammatory cytokines following CA/CPR relative to sham, regardless of housing conditions. Additional studies are necessary to determine the mechanism underlying this sex-difference in the effects of social environment on ischemia-induced neuroinflammation. However, these data highlight the importance of including both sexes in cerebral ischemia research. In sum, social environment can have a substantial influence on the pathophysiological response to global cerebral ischemia.

Disclosures: **M.M. Gaudier-Diaz:** None. **N. Zhang:** None. **A. DeVries:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.12/Y34

Topic: E.02. Neuroimmunology

Title: Development of microglia in germ free and conventionally colonized mice

Authors: *M. K. HOLDER, N. V. PETERS, A. CASTILLO-RUIZ, M. D. MOSLEY, B. CHASSAING, A. T. GEWIRTZ, N. G. FORGER, G. J. DEVRIES;
Georgia State Univ., Atlanta, GA

Abstract: The internal and external surfaces of all mammals are colonized by a diverse group of microorganisms, collectively called the microbiota. The microbiota contributes to a range of developmental processes, especially in the intestine and immune system, and the microbiota-gut-brain axis is known to influence brain development and social behavior. Furthermore, both the microbiota and microglia, the resident immune cells of the brain, have been implicated in the development of sex differences. Here, we sought to determine how rearing animals in the absence of a microbiota (i.e. germ-free; GF) would influence the microglia. As microglia

respond to signals from the peripheral immune system and GF mice often have a poorly developed immune system, there may be fewer or less activated microglia in GF mice. Alternatively, the development and maintenance of microglia may occur independently of the microbiota. To test these hypotheses, immunoreactivity (IR) of a protein constitutively expressed in microglia, ionized calcium binding adaptor molecule 1 (Iba1), was compared in neonatal (postnatal day 3; PN3), juvenile (PN21), and adult (PN56) male and female GF and conventionally colonized (CC) Swiss-Webster mice. Furthermore, we examined the effects of re-colonization of GF mice, by exposing them to gut microbiota from conventional mice at PN28. Preliminary analyses indicate that the presence of the microbiota does not influence Iba1 IR in neonatal or juvenile mice. However, we did observe some sex differences in Iba1 IR that depended on microbiota status. Juvenile female mice have greater Iba1 IR than males in the nucleus of the solitary tract (NTS), which receives visceral input via the vagus nerve, and this sex difference was also seen in CC adult females. Adult male CC mice have greater Iba1 IR than females in the paraventricular nucleus of the thalamus (PVT), which receives brainstem afferents and projects to the forebrain. Rearing animals in a GF environment prevented the sex differences in both the NTS and PVT; moreover, re-colonization did not reinstate these sex differences. These results suggest that the presence of the microbiota does not affect the expression of Iba1 neonatally, when microglial number increase markedly in many brain regions, or during the juvenile period, when microglial morphology matures. The effects of rearing in a GF environment emerge in adulthood and appear to be sex- and region-specific, indicating that early postnatal colonization by microbiota may contribute to sex differences in adulthood.

Disclosures: **M.K. Holder:** None. **N.V. Peters:** None. **A. Castillo-Ruiz:** None. **M.D. Mosley:** None. **B. Chassaing:** None. **A.T. Gewirtz:** None. **N.G. Forger:** None. **G.J. deVries:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.13/Y35

Topic: E.02. Neuroimmunology

Title: Altered brain perfusion and structure accompany experimental sepsis-associated encephalopathy: a combined magnetic resonance imaging and histology study

Authors: *J. KONSMAN, I. DHAYA, M. GRITON, G. RAFFARD, B. HIBA;
CNRS UMR 5536 RMSB / Univ. Bordeaux, Bordeaux, France

Abstract: Sepsis-associated encephalopathy (SAE) refers to brain dysfunction during systemic inflammation, which can range from mild delirium to coma. Despite its clinical relevance and poor outcome, SAE remains poorly understood even though magnetic resonance imaging (MRI) in septic patients has provided indications of cerebral vasospasms and diffuse edema. In the

present work, we combined advanced MRI modalities and post mortem histology to study the brains of the same animals 24h after induction of sepsis by cecal ligature and puncture (CLP) in an attempt to better understand the pathophysiology of SAE. Sepsis was accompanied by the classical signs of sickness behaviour, but also by reduced reflexes indicating nervous system dysfunction. Assessment of cerebral perfusion with Arterial Spin Labeling (ASL) revealed that the cortex of animals that underwent CLP received less blood relative to the whole brain compared to sham surgery. T2 MRI indicated the presence of more water in the cerebral cortex after CLP than after sham surgery. Diffusion Tensor Imaging (DTI) showed increased water diffusion parallel to the fibers of the corpus callosum after CLP as compared to sham surgery. These imaging findings indicating both functional and structural CNS changes during experimental sepsis. We are currently studying to what extent these changes are linked to perivascular prostaglandin production, blood-brain barrier breakdown and glial responses affecting water diffusion on brain sections of the same animals.

Disclosures: J. Konsman: None. **I. Dhaya:** None. **M. Griton:** None. **G. Raffard:** None. **B. Hiba:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.14/Y36

Topic: E.02. Neuroimmunology

Support: SFI/IA/1537

Title: TLX, a regulator of neural stem cell self-renewal, is required for microglial integrity in the adult mouse dentate gyrus

Authors: D. A. KOZAREVA[1], C. M. HUESTON[1], C. S. Ó'LÉIME[1], J. F. CRYAN[1], *Y. M. NOLAN[2];
[1]Dept. of Anat. and Neurosci., [2]Univ. Col. Cork, Cork, Ireland

Abstract: Tailless homolog TLX (Nr2e1) is an orphan nuclear receptor expressed primarily in the neurogenic niches of the adult mouse brain. At present, the functions attributed to the receptor are the maintenance of the stem cell pool in a proliferative and undifferentiated state, as well as regulating hippocampal neurogenesis-associated tasks of spatial learning and memory. Extracellular stimulation of both embryonic and adult hippocampal neural stem cells with pro-inflammatory cytokine IL-1β has recently been shown to detrimentally affect TLX expression. Hippocampal inflammation has been implicated in the pathology of many neurodegenerative and psychiatric disorders including Alzheimer's disease and stress-induced depression. Alterations in adult hippocampal neurogenesis have also been implicated in these disorders. Our aim is to

understand the relationship between inflammation, TLX expression and adult hippocampal neurogenesis in order to position TLX as a novel therapeutic target for hippocampal-dependent neurodegenerative disorders that have an inflammatory component. In order to explore the interaction between TLX and inflammation, we assessed microglial morphology and hippocampal architecture in mice which have a spontaneous deletion of Nr2e1 (Nr2e1-/-). Immunohistochemistry on coronal sections through the dentate gyrus of the hippocampus of BrdU-injected mice (Nr2e1-/-; Nr2e1+/-; Nr2e1+/+) was performed to compare the number of microglia (Iba1+ cells) and number of newborn neurons (DCX+ cells), across groups. The activation status of microglial cells in the dentate gyrus of these mice was also established. To determine gene expression levels of pro- and anti- inflammatory cytokines, qrtPCR analyses was carried out on hippocampal tissue of mice from each genotype. We observed a marked discrepancy in the integrity of the dentate gyri in Nr2e1-/- mice compared to wild type. Furthermore, lack of TLX expression led to reduced numbers of newly born neurons in the adult mouse dentate gyrus as well as altered microglial morphology. We are currently establishing the pro- and anti-inflammatory cytokine profile in the hippocampus of mice from each genotype. Our current findings suggest that TLX is necessary for intact neurogenic cells as well as microglial activation.

Disclosures: D.A. Kozareva: None. **C.M. Hueston:** None. **C.S. Ó'Léime:** None. **J.F. Cryan:** None. **Y.M. Nolan:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.15/Y37

Topic: E.02. Neuroimmunology

Support: NIH Grant R01-MH091424

CONACYT Grant 236296

Title: The role of estradiol in microglial phagocytosis in the developing rat cerebellum

Authors: *M. PEREZ-POUCHOULEN, M. M. MCCARTHY;
Dept. of Pharmacol., Univ. of Maryland, SOM, Baltimore, MD

Abstract: Microglia are the resident immune cells of the central nervous system and play important roles during normal development and under injury or infectious conditions in adulthood. We recently showed microglia are non-uniformly distributed in the developing cerebellum and execute phagocytosis in an age and region dependent manner that peaks during the third postnatal week of development from postnatal day (PN) 17 to 19 in the granular layer, but not in the molecular layer of the cerebellar cortex. Aromatase is the estradiol synthesizing

enzyme and we found its expression increases around PN10 in the rat cerebellum. In order to determine if endogenous estradiol synthesis is critical for microglia function we injected the aromatase inhibitor formestane (Form, 5 µg / 0.05 ml s.c.) from PN8 to PN12 and estradiol benzoate (EB, 5 µg / 0.05 ml) from PN10 to PN14 in male and female rat pups and quantified microglial morphology and the number of phagocytic cups in the cerebellar cortex at PN17. Form treatment significantly decreased by ~ 24 % the number of phagocytic cups as well as ~ 36 % the number of phagocytic microglia with thin processes. Phagocytic microglia with thick processes decreased ~ 14 % when treated with Form compared to control; however, this decrease was not significant. No regional or sex differences were found in the cerebellar cortex between the control and Form treated groups. These data indicate endogenous estradiol regulates microglial phagocytosis in the developing cerebellum and that microglial phagocytosis at PN17 might be established during the second week of postnatal development. In contrast, treatment with EB did not effect the number of phagocytic cups or phagocytic microglia with thick or thin processes in the immature cerebellum. Nonetheless, EB slightly increased the density of overall microglia (1.1-fold) compared to control. This may be an indication of: 1) a sensitive period regulating microglial phagocytosis before PN10, or 2) the EB dose used in this experiment was too low to impact phagocytosis by microglia, although it was sufficient to modify the overall microglial population. We also analyzed mRNA by qRT-PCR for both estrogen receptors (ERs) α and β during the microglial phagocytosis window. While ERα decreased (~ 66 %) by PN19 compared to PN15, only a small change was detected for ERβ (~ 14 %) indicating estradiol may regulate microglial phagocytosis via ERα. NIH Grant R01-MH091424 to M.M.M. and CONACyT Grant 236296 to M.P.P.

Disclosures: M. Perez-Pouchoulen: None. **M.M. McCarthy:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.16/Y38

Topic: E.02. Neuroimmunology

Title: Lithium stimulates complement component C3 production via GSK-3 inhibition in monocytic cells

Authors: *Z. YU[1], C. ONO[2], S. AIBA[3], Y. KIKUCHI[2], I. SORA[4], H. MATSUOKA[5], H. TOMITA[2];
[1]Tohoku Univ., Sendai, Japan; [2]Disaster Psychiatry, [3]Dermatol., [4]Biol. Psychiatry, [5]Psychiatry, Tohoku university, Sendai, Japan

Abstract: Lithium (Li), a direct inhibitor of glycogen synthase kinase 3 (GSK-3), has been widely prescribed mood stabilizer in treatment of bipolar disorder. Evidence suggests that Li

affects monocytic cells, which play pivotal roles in the innate immune systems in both the brain and peripheral tissues. Although majority of the studies into the mechanism of action of Li has been focused on neuron, astrocyte and oligodendrocyte, recent studies indicate that Li and GSK-3 inhibition also affect immune cells, such as peripheral monocytes, macrophages, monocyte-derived dendritic cells (MoDCs) and microglia. Li and GSK-3 inhibition are shown to mediate inflammation, microglial migration, monocyte-derived dendritic cells differentiation and reverse monocytic malfunction observed in neuropsychiatric diseases. Here, we surveyed molecules which take major roles in regulating these monocytic cellular functions. MoDCs treated with 1 and 5 mM Li, and microglia separated from Li-treated mice were subjected to microarray-based comprehensive gene expression analyses. Findings were validated using multiple experiments, including quantitative PCR, ELISA and immunostaining studies. We found the Li significantly increased the complement component C3 production via GSK-3 inhibition in the mouse and human monocytic cells, which suggest a potential involvement of complement system induction into neuroprotective and mood stabilizing effects, as well as non-neurologic diseases of Li treatment.

Disclosures: **Z. Yu:** None. **C. Ono:** None. **S. Aiba:** None. **Y. Kikuchi:** None. **I. Sora:** None. **H. Matsuoka:** None. **H. Tomita:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.17/Y39

Topic: E.02. Neuroimmunology

Support: The Natural Sciences and Engineering Research Council of Canada (NSERC)

Jack Brown and Family Alzheimer Disease Research Foundation

Title: The effects of voluntary exercise on neuroinflammatory status: role of monocyte chemoattractant protein 1

Authors: *A. KLEGERIS, L. J. SPIELMAN, M. ESTAKI, C. B. POINTER, S. GHOSH, D. L. GIBSON;
Dept. of Biol., Univ. of British Columbia Okanagan Campus, Kelowna, BC, Canada

Abstract: The health benefits of exercise have been well studied, and it is universally accepted that physical activity is crucial for maintaining a healthy body. One of the proposed mechanisms for these beneficial effects involve modified peripheral immune adaptations in response to exercise. Several whole-body anti-inflammatory effects have been attributed to exercise including: decrease in circulating levels of pro-inflammatory cytokines, elevation of anti-inflammatory cytokines, reduction in the number of macrophages infiltrating adipose tissue,

switching of adipose tissue macrophages from a pro-inflammatory (M1) phenotype to an anti-inflammatory (M2) phenotype, and a decrease in the expression of toll-like receptor 4 in several peripheral tissues. To date, very few studies have looked for the anti-inflammatory effects of exercise behind the blood brain barrier. We sought to bridge this information gap by testing whether physical activity has an effect on the inflammatory status of the brain. We measured the effect of voluntary wheel running on pro- and anti-inflammatory cytokine levels in the brains of female C57BL/6 mice. A key inflammatory chemokine, monocyte chemoattractant protein (MCP)-1 is upregulated during inflammatory events in the central nervous system, and it is also critically involved in the cross talk between the peripheral immune system and the immune system of the brain. Therefore, we used MCP-1 knock-out mice to investigate the role of this inflammatory mediator in regulating the immune effects of physical activity in the periphery and in the brain. We demonstrated that mice exposed to six weeks of voluntary physical activity had modified profiles of both pro- and anti-inflammatory cytokines and glial markers in the brain, compared to their sedentary counterparts. Measurement of serum cytokine concentrations showed that the changes in brain cytokine levels could not be attributed to the peripheral effects of physical activity. We propose that this modified neuroimmune response in the physically active group represents a primed immune system, as opposed to a suppressed immune system in the sedentary group. Comparison of brain cytokine profiles between sedentary and physically active MCP-1 knock-out mice revealed the key role that MCP-1 may play in physical activity-induced modulation of neuroimmune reactions. We concluded that in addition to its peripheral effects, voluntary wheel running modifies neuroimmune reactions in a MCP-1-dependent manner. The identified mechanisms could be responsible for the observed negative relationship between physically active lifestyles and risk for a number of neurodegenerative diseases.

Disclosures: **A. Klegeris:** None. **L.J. Spielman:** None. **M. Estaki:** None. **C.B. Pointer:** None. **S. Ghosh:** None. **D.L. Gibson:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.18/Y40

Topic: E.02. Neuroimmunology

Title: Expression and lipopolysaccharide-induced shedding of polysialylated proteins by NCAM-negative microglia and human THP-1 cell-derived macrophages

Authors: ***S. WERNEBURG**[1], F. BUETTNER[1], H. NEUMANN[2], M. MÜHLENHOFF[1], H. HILDEBRANDT[1];
[1]Hannover Med. Sch., Hannover, Germany; [2]Univ. of Bonn, Bonn, Germany

Abstract: Microglia are the innate immune cells of the brain that adopt distinct reactive phenotypes in response to brain injury or infection. The glycan polysialic acid (polySia) has been implicated in the regulation of microglia activation. Recently, we detected polySia in microglia obtained from mice negative for NCAM, the major polySia protein carrier (Werneburg et al. 2015, Glia 63:1240). PolySia was strictly confined to the Golgi of these cells and a glycoproteomic approach revealed the presence of polysialylated neuropilin-2, which first has been identified in dendritic cells. Now, we demonstrate Golgi-confined polySia in stem cell-derived microglia and human THP-1 cell-derived macrophages. Western blot analysis corroborates the expression of polySia-NRP2 but glycoproteomic analysis indicates the presence of a second Golgi-confined polySia protein carrier in both cell types. Activation by lipopolysaccharide (LPS), but not interleukin-4, causes cell surface translocation and a rapid release of the two polysialylated proteins in microglia and THP-1 cell-derived macrophages. Addition of metalloproteinase inhibitors prevents polySia depletion, indicating that the release is mediated by protein ectodomain shedding. Furthermore we demonstrate that polySia inhibits LPS-induced activation of nitric oxide (NO) release. Together these data suggest that shedding of polysialylated proteins contributes to a negative feedback regulation of LPS-induced activation of microglia and possibly macrophages.

Disclosures: S. Werneburg: None. **F. Buettner:** None. **H. Neumann:** None. **M. Mühlenhoff:** None. **H. Hildebrandt:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.19/Y41

Topic: E.02. Neuroimmunology

Support: NIDCD R01 DC010154

Title: Identification of macrophage projections in the organ of Corti of the cochlea

Authors: *B. HU, W. YANG, R. R. VETHANAYAGAM;
Ctr. Hearing & Deafness, State Univ. Buffalo, Buffalo, NY

Abstract: The cochlea was traditionally considered as an immune privileged organ due to the presence of the blood-labyrinth barrier. However, recent studies have documented strong immune activities under both steady-state and disease conditions. In the cochlea, the organ of Corti houses sensory cells, which are vulnerable to stresses. Sensory cell damage is known to provoke the immune activity of cochlear macrophages. However, cochlear macrophages are commonly absent in the organ of Corti. At present, it not clear how macrophages in other anatomic sites of the cochlea detect immune signals from the organ of Corti. Here, we report the

finding of immune cell projections in the organ of Corti. The cochleae of C57BL/6J mice were immunolabeled for CD45, a receptor tyrosine phosphatase presenting on all hematopoietic/bone marrow derived leukocytes. We found CD45 positive cells in the spiral ligament, the spiral limbus, and the scala tympani side of the basilar membrane. In addition, we found CD45 positive structures in the scala media side of the basilar membrane. These structures had an irregular shape. They were located along the junction between Claudiu's cells and Hensen's cells, and were more visible in the apical and middle cochlear turns. High magnification view of confocal images showed that these structures had macrophage phenotypes including small projections on the surface and vacuoles in the cytoplasm. To provide further evidence for the nature of macrophages, we stained the tissues for F4/80, a macrophage marker protein, and found strong immunoreactivity in these structures. Surprisingly, we did not find nuclei within these structures, suggesting that these structures are not individual macrophages, but macrophage projections from neighboring tissues. We revealed two possible sources of the projections: one from the macrophages beneath the basilar membrane and the other from the macrophages in the lateral wall. We suspect that these projections enable neighboring macrophages to detect immune signals and participate in the immune activity in the organ of Corti.

Disclosures: B. Hu: None. **W. Yang:** None. **R.R. Vethanayagam:** None.

Poster

345. Neuroimmunology: Regulatory Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 345.20/Y42

Topic: E.02. Neuroimmunology

Support: HL028785

HL074011

Grant-in-Aid for JSPS Fellows

Title: Protection of acute kidney injury by electrical vagal nerve stimulation: pathways and potential mechanisms

Authors: *C. ABE[1], T. INOUE[2], D. L. ROSIN[2], M. D. OKUSA[2], P. G. GUYENET[1]; [1]Pharmacol., [2]Nephrology, Univ. of Virginia, Charlottesville, VA

Abstract: The vagal nerve (VN) regulates the immune system (Cholinergic Anti-inflammatory Pathway). For example, electrical stimulation of VN efferents suppresses lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF-α) release from macrophages. Here we show that VN stimulation reduces kidney ischemia/reperfusion injury (IRI) in mice and rats and we explore some of the mechanisms responsible for this protective effect. Electrical stimulation of

VN efferents or afferents in mice (50 μA, 1 ms, 5 Hz, 10 min) was performed 24 h before induction of renal ischemia (clamping both renal artery and vein for 26 min). Stimulation of VN efferents or afferents equally suppressed plasma creatinine 24 hours after kidney IRI. The C1 neurons are lower brainstem neurons that activate both sympathetic and VN efferents (Abbott et al., Eur J Neurosci, 2014). C1 neurons were unilaterally transduced to express ChR2 in dopamine-beta-hydroxylase Cre mice using a Cre-dependent AAV2. Selective optogenetic stimulation of the C1 neurons in conscious mice attenuate the rise in creatinine elicited by kidney IRI to the same extent as VN nerve stimulation. In rats, selective stimulation of VN efferents (150 μA, 1 ms, 5 Hz, delivered under anesthesia 24 hs before kidney IRI) also reduced the rise in creatinine present 24 h after kidney ischemia (45 min). VN efferents stimulation had no effect on the splenic sympathetic nerve mass activity whereas VN afferents stimulation (150 μA, 1 ms, 1 Hz) evoked a robust response in this nerve consisting of two distinct activation peaks. This evidence suggests that the protective effect of VN efferents stimulation might not be mediated by splenic sympathetic nerve activation but via other immune organs innervated by the VN e.g. the thymus. We confirmed that the thymus receives VN efferents via a collateral branch of the recurrent nerve and we showed that thymectomy (7 days before VN stimulation) prevented the beneficial effect of efferent VN stimulation on kidney IRI as judged by the creatinine level 24 hs after renal ischemia. In conclusion, stimulation of either VN efferents or afferents protects the acute kidney injury. C1 cell stimulation is equally effective, probably because these neurons activate both VN and sympathetic efferents. The immunosuppressive effect of VN efferent stimulation may be mediated by the thymus and this organ may provide a humoral link to the spleen and the previously described Cholinergic Anti-inflammatory Pathway.

Disclosures: C. Abe: None. **T. Inoue:** None. **D.L. Rosin:** None. **M.D. Okusa:** None. **P.G. Guyenet:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.01/Y43

Topic: E.02. Neuroimmunology

Title: Microglia polarization: a common paradigm spanning from neuro-oncology to infectious diseases

Authors: *L. LISI[1], E. LAUDATI[2], C. DELLO RUSSO[3], P. NAVARRA[1];
[1]Catholic Univ. Med. Sch., Rome, Italy; [2]Catholoic Univ. Med. Sch., Roma, Italy; [3]catholic Univ. Med. Sch., Roma, Italy

Abstract: We have investigated microglia polarization in the framework of microglia interactions with primary brain tumors. In a series of *in vitro* experiments, we have characterized

the influence of glioma-soluble factors on microglial function, comparing the effects of media harvested under basal conditions with those of media obtained after inducing a pro-inflammatory activation state in glioma cells. Microglia exposed to basal glioma-derived factors (a condition resembling the early stage of pathology), shows increased M2b polarization status and up-regulation of IL-10 only. At variance, when exposed to activated glioma-derived factors (a condition mimicking the late stage of pathology), microglia presents as a mixture of polarization phenotypes (M1 and M2a/b), with up-regulation of iNOS, arginase and IL-10. In this paradigm, the inhibition of mTOR polarizes glioma-activated microglial cells towards the M1 phenotype, thus preventing the induction of the M2 status that would promote tumor growth. Investigations are currently underway on 54 surgical specimens of glioblastoma multiforme to confirm the influence of brain tumor exposure on microglia polarization. An apparently unrelated line of research in our lab was addressed to investigate the effects of antiretroviral drugs (ARVs) exposure on microglia cultures, seeking for putative mechanism of drug neurotoxicity. We found that certain RRTIs and PIs increased NO production with a mechanism independent from iNOS induction. Rather, these agents increased the availability of the iNOS substrate L-arginine by blocking arginase, a well-established marker of M2 polarization. Thus, the investigation of microglia polarization markers turned out to be a common background linking studies on the most different patho-physiological conditions involving the CNS.

Disclosures: **L. Lisi:** None. **E. Laudati:** None. **C. Dello Russo:** None. **P. Navarra:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.02/Y44

Topic: E.02. Neuroimmunology

Support: This work was supported by the Peter Deane Trust (KB)

Title: CCL2-CCR2 mediated monocyte trafficking into the VSV-infected brain is under circadian control

Authors: ***K. GAGNIDZE**, K. H. HAJDAROVIC, K. BULLOCH;
Neuroimmunology and Inflammation Program, Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY

Abstract: In the mammalian organism many immune functions are under circadian control. We have recently shown that circadian time of infection has a profound effect on the survival of mice in a vesicular stomatitis virus (VSV) induced encephalitis model. Specifically, mice infected at zeitgeber time 0 (ZT0) were more susceptible to LD_{50} dose of the virus than mice infected at ZT12. The poor survival of ZT0 mice was accompanied by higher accumulation of

$CD45^{hi}/CD11b^{hi}$ cell to the site of infection, the olfactory bulb (OB), 4 days post-infection (dpi). Further analysis revealed that this cell population comprises inflammatory monocytes, bone marrow-derived cells characterized by the expression of $Ly6c^{hi}/CCR2^{+}$, which egress into the bloodstream and migrate to the site of infection where they can differentiate into macrophages and dendritic cells (DC). Signaling through chemokine receptor, CCR2, is known to be essential for the migration of monocytes to the site of infection where they help clear pathogens. However, if left unregulated, monocytes can also contribute to the damage in chronic inflammatory diseases. In the current study we tested the hypothesis that *CCL2-CCR2 mediated migration of inflammatory monocytes is under circadian control and contributes to poor survival in ZT0 mice in VSV-induced encephalitis model*. Analysis of CCL2 mRNA in steady-state mouse OB revealed the rhythmic pattern of expression with higher levels observed at ZT0 compared to ZT12. To identify the cellular source of CCL2 in the OB, we measured mRNA in sorted microglia (MG, $CD45b^{int}/CD11b^{+}/CD11c^{-}$), brain DC ($CD45^{int}/CD11b^{+}/CD11c^{+}$) and monocytes ($CD45^{hi}/CD11b^{+}/CD11c^{+}$), and found that at 4 dpi VSV, brain-resident MG express significantly higher levels of CCL2 at ZT0, whereas bDC and monocytes display higher levels of CCL2 at ZT12. Next we examined whether egress of monocytes into the bloodstream, which depends on CCL2-CCR2 signaling, is also under circadian regulation. We found a higher number of $Ly6c^{hi}/CCR2^{+}$ monocytes present in the blood at ZT0 compared to ZT12 in non-infected animals. To test whether CCR2-mediated trafficking of inflammatory monocytes into the brain contributes to VSV neuropathology, we blocked CCR2 using systemic injections of antagonist (RS504393) in VSV-infected mice 0, 1 and 2 dpi. Blocking CCR2 signaling within the first three days of VSV infection improves survival in both, ZT0 and ZT12 groups, and nullifies circadian effect on survival. In summary, this data demonstrates circadian regulation of CCL2-CCR2 mediated monocyte trafficking to the brain, thus contributing to VSV-induced neuropathology and mortality.

Disclosures: K. Gagnidze: None. **K.H. Hajdarovic:** None. **K. Bulloch:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.03/Z1

Topic: E.02. Neuroimmunology

Title: Chronic unpredictable mild stress alters the metabolism of carbohydrates and lipids in rats by oxidative stress and systemic inflammation

Authors: A. L. LÒPEZ-LÒPEZ[1], *M. ARTEAGA-SILVA[2], F. J. ALARCÓN-AGUILAR[1], M. C. ESCOBAR-VILLANUEVA[1], H. BONILLA-JAIME[1];

[1]Univ. Autonoma Metropolitana, Mexico, D.F, Mexico; [2]Univ. Autónoma Metropolitana-Iztapalapa, Méxcio, Mexico

Abstract: Physiological stress is a risk factor for many pathologies that are considered priority health problems. It has been suggested that chronic glucocorticoid and catecholamine circulation, released during stress response, activate damage mechanisms, which at long term producing metabolic alterations that can be associated to oxidative stress and inflammation. However, the consequences of stress in animal models in periods longer than 40 days not been explored. The goal of this work was determining if the chronic unpredictable mild stress (CUMS) induces metabolic alterations due to modifications in the redox state and the inflammatory profile of rats at 20, 40, and 60 days of stress. CUMS consisted in randomly exposing the animals to different stressors. The effects of CUMS on corticosterone, body weight, glucose tolerance, triglyceride, cholesterol levels and insulin were evaluated. In liver and pancreas was determined reduced glutathione (GSH), lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC) and protein oxidation. Likewise, cytokine serum levels (IL-6, TNF-α, IL-1β, and IL-10) were determined. Results show that CUMS decreased weight gain after of 40 days of stress, while at 60 days the rats presented glucose intolerance. A reduction was observed in cholesterol levels at 40 and 60 days, and triglycerides decreased at 60 days. Insulin levels were elevated only at 20 days. In liver and pancreas, GHS levels were decreased from day 40, while protein lipid peroxidation and protein oxidation were increased. This is the first work where pancreas redox state under chronic stress conditions is reported. Liver TAC was constant while in pancreas it was reduced. Regarding inflammation markers, an increase in TNF-α, IL-1β, and IL-6 levels was observed, while IL-10 levels were reduced due to CUMS, generating a systemic inflammation state. Corticosterone levels remained high and constant up to the end of the experimental period at 60 days. These data suggest an association between chronic stress and metabolic alterations that may gradually alter carbohydrate and lipid metabolism. CUMS consequences at day 60 suggest that oxidative stress and inflammation can contribute the development of chronic degenerative diseases, like cardiovascular disease and diabetes mellitus. CUMS is an animal model that in addition to avoiding habituation, it activates damage mechanisms, like oxidative stress and low-grade chronic inflammation, which allows the study of physio-pathological stress aspects in prolonged time periods, of at least 60 days.

Disclosures: **A.L. Lòpez-Lòpez:** None. **M. Arteaga-Silva:** None. **F.J. Alarcón-Aguilar:** None. **M.C. Escobar-Villanueva:** None. **H. Bonilla-Jaime:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.04/Z2

Topic: E.02. Neuroimmunology

Support: Peter Deane Trust

Title: Apobec1 ko mice manifest neuropsychological defects

Authors: *D. C. COLE[1,2], K. H. HAJDAROVIC[2], F. PAPAVASILIOU[3], K. BULLOCH[2]; [1]Neuroimmunology, [2]Neuroimmunology and Inflammation Program, Lab. of Neuroendocrinology, [3]Lymphocyte Biol., The Rockefeller Univ., New York, NY

Abstract: Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (Apobec1) is an mRNA editing enzyme that induces C to U transitions in specific mRNAs. It has been implicated in the host immune response, the regulation of cell identity, the development of cancer, and lipid metabolism regulation. Apobec1 and its cofactors have also been found to be differentially expressed in certain cell types including monocytic cells of the CNS. The cell type specific regulation of Apobec1 and its cofactors in the brain indicates that it could be a potent regulator of immune function. Our analysis of immune mediators in the steady state Apobec1 KO mouse showed that several markers of inflammation were up-regulated in the cortex and hippocampus, areas associated with memory and learning, by three months of age. Given that chronic inflammation is thought to underlie many cognitive and neurological dysfunctions we examined the behavior of male Apobec1 KO mice at one year old, 9 months old, and 3 months old. When tested for anxiety using an open field test or elevated plus maze, we found that Apobec1 KO mice are far more anxious than their male wild type litter mates. This effect is seen to increase with age: one year old mice spent less time in the open arms of the plus maze and the center of the open field as compared to younger mice. When tested for depression using the splash test, the KO mice were found to have a severe depressive phenotype, but were not found to be anhedonic. This effect did not exhibit age related increases in severity, unlike the anxiety phenotype. When assayed for memory deficits using novel object recognition the KO mice displayed significant memory impairment that progressed with age. These findings demonstrate that Apobec1 mediated mRNA editing plays a regulatory role on inflammatory mediators in the brain; and that loss of Apobec1 mediated RNA editing as seen in the KO animals can contribute to neuropsychological and degenerative brain diseases.

Disclosures: D.C. Cole: None. **K.H. Hajdarovic:** None. **F. Papavasiliou:** None. **K. Bulloch:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.05/Z3

Topic: E.02. Neuroimmunology

Support: 1R01AI093718

5R37GM043880

1U19AI077435

Title: Sphingosine 1-phosphate (S1P) dependent c-IAP2 mediated ubiquitination of IRF-1 is essential for IL-1 induced CCL5 and IP-10 production

Authors: *K. HARIKUMAR[1], J. YESTER[2], M. SURACE[2], C. OYENIRAN[2], M. PRICE[2], W.-C. HUANG[2], N. HAIT[2], J. C. ALLEGOOD[2], A. YAMADA[2], R. BHARDWAJ[2], K. TAKABE[2], S. MILSTIEN[2], S. SPIEGEL[2], T. KORDULA[2];
[1]Cancer Res., Rajiv Gandhi Ctr. For Biotech., Thiruvananthapuram, India; [2]Virginia commonwealth Univ., Richmond, VA

Abstract: Sphingosine-1-phosphate (S1P) and sphingosine kinase 1, one of the isoenzymes that produce the potent bioactive lipid mediator S1P, have long been implicated in the actions of pro-inflammatory cytokines. Interleukin 1 (IL-1), a quintessential pro-inflammatory cytokine that plays a major role in many inflammatory diseases, enhances the production of various chemokines, including RANTES (CCL5) and IP-10 (CXCL10), which exacerbate inflammation. However, the regulatory mechanisms regulating production of these chemokines have largely remained unclear. Here, we show that IL-1-mediated effects require activation of yet another transcription factor, IRF-1 (interferon regulatory factor-1). Interestingly, IL-1-induced IRF-1 activation required its K63-linked polyubiquitination that was mediated by cIAP2 and depended on the presence of S1P that acted as a co-factor for cIAP2 E3 ligase activity. The IL-1-mediated RANTES and IP-10 production was abolished in IRF1-/- and cIAP2-/- MEFs as well as IRF1-/- mice. In addition, RANTES and IP-10 levels were markedly diminished in the serum of IL-1-treated SphK1-/- mice. This study provides a mechanistic explanation for the key role of SphK1 and intracellular S1P in IL-1-mediated chemokine production and identifies cIAP2 as a novel intracellular target of S1P. Further our findings may in part explain the diminished susceptibility of Irf1-/- mice to autoimmune diseases, such as collagen-induced arthritis and mouse models of multiple sclerosis that are in part IL-1-dependent. This work was supported by grants from the National Institute of Health 1R01AI093718 (to T.K.), 5R37GM043880 and 1U19AI077435 (both to S.S.)

Disclosures: **K. Harikumar:** None. **J. Yester:** None. **M. Surace:** None. **C. Oyeniran:** None. **M. Price:** None. **W. Huang:** None. **N. Hait:** None. **J.C. Allegood:** None. **A. Yamada:** None. **R. Bhardwaj:** None. **K. Takabe:** None. **S. Milstien:** None. **S. Spiegel:** None. **T. Kordula:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.06/Z4

Topic: E.02. Neuroimmunology

Support: AIHS-CRIO Team program

Title: Chondroitin sulfate proteoglycans in multiple sclerosis

Authors: *E. STEPHENSON, J. A. ROGERS, M. B. KEOUGH, M. MISHRA, V. W. YONG; Univ. of Calgary, Calgary, AB, Canada

Abstract: Multiple sclerosis (MS) is a chronic inflammatory and degenerative disease of the central nervous system, presenting with profound destruction of myelin and axons that form focal areas of demyelination. MS also presents with changes in the extracellular matrix, a network of molecules involved in maintaining brain architecture. The extracellular matrix molecules observed at the border of active demyelinating lesions in MS are the chondroitin sulfate proteoglycans (CSPGs) (Sobel and Ahmed J Neuropathol Exp Neurol 2001). CSPGs may have the ability to promote activation of immune cell subsets, migration of immune cells into the CNS, and killing of neurons. Thus, we hypothesize that interfering with CSPG production in MS and in an animal model of MS, experimental autoimmune encephalomyelitis (EAE), will improve outcomes. The purpose of this work was to investigate the ability of CSPGs to influence the immune system, and characterize changes in CSPGs over the course of EAE and MS. We also tested whether a CSPG-targeting glucosamine derivative can affect inflammation, neuropathology, and clinical disease scores in EAE. To study the role of CSPGs in T cell proliferation we used *in vitro* proliferation assays, where T cells were exposed to CSPGs with or without anti-CD3/anti-CD28 co-stimulation. Cell survival was assessed by propidium iodide staining and flow cytometry. EAE was induced by immunization with the immunogenic portion of the myelin protein myelin oligodendrocyte glycoprotein (MOG35-55) in C57Bl6 mice. Immunohistochemistry and real-time polymerase chain reaction were used to determine changes in CSPG protein and transcript levels, respectively. MS tissues were also investigated for CSPG expression with immunohistochemistry. Current results show that CSPGs promoted proliferation of T cells in culture in an activation-dependent manner. At peak severity of EAE in mice, we observed an upregulation of transcripts and protein of the CSPG member versican. Other CSPG members were not significantly altered. Versican was also upregulated in MS tissue, particularly in the vicinity of inflammatory perivascular cuffs. EAE mice treated at onset or peak with a CSPG-lowering glucosamine derivative had reduced EAE severity and reduction of inflammatory transcripts. Thus, CSPGs, such as versican, may promote inflammatory disease in MS and EAE. These results emphasize that reducing CSPGs may prove useful in pathologies such as MS, where the immune system plays a role in central nervous system damage.

Disclosures: E. Stephenson: None. **J.A. Rogers:** None. **M.B. Keough:** None. **M. Mishra:** None. **V.W. Yong:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; AIHS-CRIO Team program.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.07/Z5

Topic: E.02. Neuroimmunology

Support: This work was supported by the Peter Deane Trust (KB).

Title: Strategic utilization of SGs by VSV for replication and survival in cultured microglia but not neurons

Authors: *Y. CHUNG[1], D. C. COLE[1], N. F. PAPAVASILIOU[2], K. BULLOCH[1]; [1]Rockefeller Univ., New York, NY; [2]Lab. of Lymphocyte Biol., The Rockefeller Univ., The Rockefeller University, NY

Abstract: Stress granules (SGs) are dynamic non-membranous cellular structures composed of proteins and translationally silenced mRNA that are formed under stress conditions. Emerging evidence shows that certain viruses can subvert RNA granule function and co-opt SGs to increase their survival and proliferation. In the present study, we examined how the maintenance and function of SGs is regulated by vesicular stomatitis virus (VSV) infection in different neural cell types. Our *in vitro* studies show that VSV infection induces the formation of distinct SG types (TIA-1, G3BP and TDP-43 positive SG vs TIA-1, G3BP positive and TDP-43 negative SG) in E^tC cerebellar granule neuronal cells and BV2 microglial cells, respectively. In the BV2 microglia which form noncanonical SGs, viral replication and cell death are increased. Whereas, in the E^tC neuronal cell line that form canonical SGs viral replication is diminished and survival is high compared to the microglia. Moreover, the formation of these compositionally different types of SG is mediated by cytoplasmic localization of TDP-43 and cell-type specific regulation of TIA-1 and TDP-43. We also show that decreased TIA-1 expression induces up regulation of Apolipoprotein B mRNA editing enzyme complex (Apobec1) and its cofactors (A1CF and RBM47) in VSV-infected BV2 microglial cells, but not in E^tC neuronal cells. This increase in Apobec1 correlates with the dampening of the BV2 intracellular viral protection mechanism. These results suggest that VSV infection can manipulate the stress response mechanism and RNA metabolism of specific neural cell types to promote viral replication. This work was supported by the Peter Deane Trust (KB).

Disclosures: Y. Chung: None. **D.C. Cole:** None. **N.F. Papavasiliou:** None. **K. Bulloch:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.08/Z6

Topic: E.02. Neuroimmunology

Support: CONACYT fellowship

Title: Cytochrome P450 2J3 and 2C11 regulation in a LPS-induced model of neuroinflammation in astrocytes

Authors: *C. NAVARRO-MABARAK, R. CAMACHO-CARRANZA, J. J. ESPINOSA-AGUIRRE;
Medicina Genómica y Toxicología Ambiental, Inst. de Investigaciones Biomédicas, UNAM, DF, Mexico

Abstract: Introduction: The development and progression of several neurodegenerative and neuropsychiatric illnesses have been related to inflammatory processes in the Central Nervous System (CNS). The cytochrome P450 (CYP) epoxygenases and its metabolites, the epoxyeicosatrienoic acids (EET) have been proposed as important therapeutic targets for the treatment of both systemic and organ specific inflammatory processes, due in part to its potent anti-inflammatory activity. However, little has been described about the regulation of these enzymes during inflammation in the CNS. It has been reported that the expression of some CYP can be modified by pro-inflammatory cytokines such as IL-6, IL-1b and TNF-a. Cytokine-mediated down regulation of some CYP has been related to NF-kB binding to the promoter region of its genes. Our goal is to elucidate whether an inflammatory process developed in astrocytes is able to modify CYP2J3 and 2C11 expression and the mechanism by which this process is carried out. **Materials and methods:** Rat brain primary astroglial cultures were obtained from the cortex of newborn Wistar rats. Immunocytochemical identification of astrocytes was performed. Cultures were treated with 100 ng/ml LPS, 5 ng/ml TNF-a, 100 ng/ml LPS + 1 ng/ml IMD-0354 (selective NF-kB inhibitor) or 5 ng/ml TNF-a + 1 ng/ml IMD-0354. CYP2J3 and 2C11 mRNA expression was determined by qRT-PCR. CYP2J3 protein levels were determined by Western blot. **Results:** The addition of LPS and TNF-a to astrocytes cultures caused a decrease in CYP2J3 and 2C11 mRNA expression. Concurrent addition of IMD-0354 to the cultures caused an inhibition of the observed effect on mRNA expression. CYP2J3 protein levels were also decreased after LPS treatment. **Conclusions:** The inflammatory process triggered by the addition of LPS to astrocytes cultures is able to down-regulate CYP2J3 and CYP2C11 mRNA expression and CYP2J3 protein levels. LPS mediated down-regulation of CYP2J3 and 2C11 expression may be due in part to the production of pro-inflammatory cytokines like TNF-a, since this cytokine is also able to down-regulate CYPs mRNA independently of LPS addition. Transcription factor NF-kB may play an important role in LPS/TNF-a mediated down-regulation of CYP2J3 and 2C11 since its inhibition by IMD-0354 reversed the observed effects.

Disclosures: C. Navarro-Mabarak: None. **R. Camacho-Carranza:** None. **J.J. Espinosa-Aguirre:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.09/Z7

Topic: E.02. Neuroimmunology

Support: FAPERJ-Brasil

CAPES-Brasil

CNPq-Brasil

UFF- Proppi

Title: Effects of intravitreal treatment with IL-4 or IL-6 recruits different signaling molecules to modulate retinal afferent distribution within superior colliculus

Authors: *P. CAMPELLO-COSTA[1], G. MENEZES[2], V. G. GOULART[2], A. C. F. MELIBEU[2], C. A. SERFATY[2];
[1]Federal Fluminense Univ., Niterói, Brazil; [2]Federal Fluminense Univ., Niteroi, Brazil

Abstract: Interleukins, classical immune molecules, can induce many effects in different areas of the central nervous system (CNS), including retina. Interleukin- 4 (IL-4) and interkeukin-6 (IL-6), are classically known as anti-inflammatory and pro-inflammatory cytokines, respectively. Some studies have demonstrated effects of both cytokines in different aspects of CNS development such as survival of neurons, proliferation, differentiation and synaptic plasticity among others. The aim of the present research is to investigate the signaling pathway activated after intravitreal injection of IL4 or IL6 in the retina. We also analyse the effect of exogenous application of IL-4 or IL-6 upon the development of visual circuits, using the retinotectal pathways of rodents as a model. Lister Hooded rats were injected with either IL-6 or IL-4 in the right eye at PND10. Control matched-group received a PBS injection. At PND11 or PND14, animals were euthanized. Retinas were prepared for western blot or immunohistochemistry procedures to analyze both different intracellular mediators and glial markers. Nissl staining was performed in order to evaluate retinal cytoarchitecture after intraocular injection. Alternatively, a group of animals treated at PND11, received HRP injection in the left eye at PND14 to assess the distribution of retinal afferents into the superior colliculus after cytokines treatment. The thickness of retinal layers was similar between PBS- and cytokines treated groups and no pyknotic nuclei or inflammatory profile cells were detected with Nissl staining. Also, no difference was observed in Iba1 levels in all groups. Concerning astrocyte marker, IL-4 treated animals presented a lower level of GFAP content compared to control (PBS-treated rats) while IL-6 produced no change in the level of GFAP. The data from biochemical analysis revealed that IL-4 treated animals presented a transient increase in pSTAT6 24h after injection and a more sustained increase in pERK levels which was detected at PND11 through PND14. IL-6 treated animals presented an increase in pSTAT3 levels. No difference was observed in pAKT content

in any group. Both cytokines lead to a disruption in the uncrossed retinotectal connections of the intact eye. Our data show that exogenous *in vivo* treatments with IL-4 or IL-6 modulate different intracellular pathways in the retina, without inducing an inflammatory response. Moreover these modifications seem to trigger a different pattern of retinal activity, which in turns could be responsible for the reorganization of retinal afferent in the target.

Disclosures: P. Campello-Costa: None. **G. Menezes:** None. **V.G. Goulart:** None. **A.C.F. Melibeu:** None. **C.A. Serfaty:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.10/Z8

Topic: E.02. Neuroimmunology

Support: NIH Grant R01 MH090127

NIH Grant T32 NS 082145

NIH Grant 1F31 MH102070-01A1

Title: Neurotoxic kynurenine metabolism modulates microglial activity following lipopolysaccharide challenge

Authors: *A. M. DUGAN[1,2], J. M. PARROTT[1,2], J. N. DELGADO[1], J. C. O'CONNOR[1,2,3]; [1]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San A, San Antonio, TX; [2]Sch. of Med., Ctr. for Biomed. Neurosci., San Antonio, TX; [3]South Texas Veterans Hlth. Syst., Audie L. Murphy VA Hosp., San Antonio, TX

Abstract: The kynurenine pathway (KP) of tryptophan metabolism is the major tryptophan degradation pathway in the body, and disruption of KP metabolic balance within the brain has been implicated in both neurodegenerative and neuropsychiatric disease. Inflammation in the brain skews KP metabolism toward increased production of oxidative and excitatory metabolites, but the role of microglia in this process remains poorly understood. To investigate this further, BV-2 murine microglia were challenged *in vitro* with lipopolysaccharide (LPS), a component of gram-negative bacteria cell wall. LPS treatment up-regulated pro-inflammatory cytokine and inducible nitric oxide synthase mRNA expression. Extracellular accumulation of nitrite, an indirect index of nitric oxide production, was significantly increased 24 hours following LPS challenge. Additionally, mRNA expression of the rate limiting enzymes for the formation of oxidative/excitatory KP metabolites, indoleamine-2,3-dioxygenase (IDO)-1 and kynurenine monooxygenase (KMO), was significantly increased 6 hours after LPS. Accumulation of kynurenine and the excitotoxic metabolite quinolinic acid was also increased in the media of

microglia 24 hours after LPS. To determine whether synthesis of oxidative/excitatory KP metabolites contributes to microglial activation, BV-2 cells were treated with the KMO inhibitor Ro 61-8048 at the same time as LPS challenge. Ro 61-8048 attenuated the production of extracellular nitrite and up-regulation of KMO and tumor necrosis factor-α, but not other pro-inflammatory gene targets following LPS challenge. Further, this experiment was repeated in primary microglia isolated from neonatal C57BL6/J wild type (WT) and KMO knockout (KMO-/-) mice. The KMO-/- genotype was sufficient to reduce nitrite accumulation with or without LPS treatment. There was also a main effect of LPS in both WT and KMO-/- microglia. These data indicate that microglia are likely playing an important role in skewing KP metabolism in the brain during inflammatory conditions, and oxidative/excitatory KP metabolites modulate microglial activity during immune challenge.

Disclosures: **A.M. Dugan:** None. **J.M. Parrott:** None. **J.N. Delgado:** None. **J.C. O'Connor:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.11/Z9

Topic: E.02. Neuroimmunology

Support: NIDA NIH Grant DA007908

Title: Characterization of the role of the cannabinoid receptors on dendritic cell antigen presenting function

Authors: *J. SUAREZ MARTINEZ[1], R. B. CRAWFORD[2], N. E. KAMISNKI[2];
[1]Michigan State Universirty, East Lansing, MI; [2]Pharmacol. and Toxicology, Michigan State Univ., East Lansing, MI

Abstract: Cannabis is the most commonly used illicit drug in the United States. Δ9-Tetrahydrocannabinol (THC) is the principle psychoactive component in cannabis sativa causing well-known analgesic and behavioral effects. Additionally, THC has immunosuppressive effects, in part, mediated through cannabinoid receptor 1 (CB1) and 2 (CB2) ligation. Simultaneous deletion of CB1 and CB2 resulted in exacerbated immune reactivity to influenza infection. Dendritic cells (DCs) were identified to play a role in influenza induced immunopathology in cannabinoid receptor null mice (CB1-/-/CB2-/-). The objective of the present study is to characterize the role of the cannabinoid receptors (CBRs) on bone marrow DC development and function. Bone marrow cells extracted from femurs and tibias of C57Bl/6 (wild type, WT) and CB1-/-/CB2-/- mice were stimulated with lipopolysaccharide (LPS) and stained for DC by expression of CD11c and CD11b. Our results indicate that the percent of DC populations,

determined by CD11c expression, composes ~38% of freshly isolated bone marrow cells in WT mice and ~48% in CB1-/-/CB2-/- mice. We also found MHC I expression in CD11c+ DCs was not significantly different between WT and CB1-/-/CB2-/- mice. Interestingly, bone marrow cells isolated from CB1-/-/CB2-/- mice elicited a CD8+ T cell response in the absence of LPS stimulation. The expression of antigen-bound MHC I complexes on the surface of DCs was determined.. After 24 hours of incubation in the presence or absence of LPS, cells were washed thoroughly, pulsed with SIINFEKL peptide for two hours, washed and stained with an MHC I-SIINFEKL complex antibody. A significant increase in antigen-MHC I complexes in the surface of DCs from CB1-/-/CB2-/- mice was not observed in comparison with WT. The process of naïve T cell activation by DCs encompasses three signals. The first signal is the ligation of the T cell receptor to peptide-bound MHC molecules on DCs. The second signal can be either co-stimulatory that leads to T cell proliferation or co-inhibitory that debilitates the T cell response. The third signal, usually mediated by DC-derived cytokines, promotes the development into a specific effector or toloregenic T cell (1,2). Our results have demonstrate that the enhanced CD8+ T cell response observed is not due to an increase in MHC I expression or antigen loading to the molecule. Future studies will explore whether there is enhanced co-stimulation or soluble factor secretion in the bone marrow DCs of CB1-/-/CB2-/- mice.

Disclosures: J. Suarez Martinez: None. **R.B. Crawford:** None. **N.E. Kamisnki:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.12/Z10

Topic: E.02. Neuroimmunology

Support: Myelin Repair Foundation Research Grant

Title: Pathologic T cell cytokines have both beneficial and deleterious effects on oligodendrocyte lineage cells

Authors: *A. P. ROBINSON[1], K. LYMAN[2], Y. HWANG[1], W. LINDSTROM[1], J. RODGERS[1], S. D. MILLER[1];
[1]Microbiology-Immunology, [2]1.Davee Dept. of Neurol. and Clin. Neurosciences and the Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: The acute multiple sclerosis (MS) lesion is a highly inflammatory environment characterized by demyelination and oligodendrocyte loss. A clear pathologic role for inflammatory T cell subsets (Th1 and Th17) and cytokines (IFNγ, IL-17, GM-CSF) in MS and the animal model EAE have been demonstrated. Evidence for direct cytokine-induced effects on oligodendrocyte progenitor cells (OPCs) that undertake the reparative process of remyelination

has been less forthcoming. We stimulated OPCs in culture with IFNγ, IL-17, or GM-CSF and assessed their viability, proliferation, and maturation by histological and molecular techniques. The prototypical Th1 cytokine IFNγ had a deleterious effect directly inducing cell death in pure cultures of OPCs. Surprisingly the more recently identified Th17 cytokines IL-17 and GM-CSF had mixed effects: IL-17 stimulated OPC maturation without a loss in cell viability, whereas GM-CSF inhibited OPC maturation *in vitro* also without affecting viability. Additionally IL-17 and GM-CSF induced unique chemokine and receptor expression changes suggesting varied roles in OPC migration as well. These results suggest that inflammatory cytokines despite contributing to aberrant immune function in the lesion can have unique effects on remyelination and repair. A full characterization of pro- and anti-inflammatory cytokine effects on oligodendrocytes and remyelination may provide clues for more targeted therapeutic strategies for MS.

Disclosures: A.P. Robinson: None. **K. Lyman:** None. **Y. Hwang:** None. **W. Lindstrom:** None. **J. Rodgers:** None. **S.D. Miller:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.13/Z11

Topic: E.02. Neuroimmunology

Support: The Peter Deane Trust (KB)

Title: RNA editing enzyme Complex APOBEC1 and its cofactor in monocytes/microglia linked to the sexually dimorphic response to VSV encephalitis

Authors: *K. HAJDAROVIC[1,2], K. GAGNIDZE[1], D. C. COLE[1], K. BULLOCH[1]; [1]McEwen, [2]Neuroimmunology and Inflammation Program, The Rockefeller Univ., New York, NY

Abstract: Sex affects outcomes of viral infection both in the brain and in the periphery, with females often surviving better than males, who have less robust responses or suffer more damage from inflammation. However, the increased resistance to infection comes at a cost, as females are more likely to be affected by autoimmune disease than males. In mice, the response to vesicular stomatitis virus (VSV) has been known to be sexually dimorphic. However, a mechanism for phenomenon has not yet been elucidated. Survival studies on sexually immature and sexually mature mice intranasally infected with an LD50 dose of VSV show that the sex effect may be related to the production of female gonadal hormones, as there is no difference in survival between male and female mice at 4 weeks of age. Flow cytometry analysis done on the olfactory bulbs of mice infected in the same manner show that females in proestrus have higher

number of microglia (MG, CD45int/ CD11bhi/CD11c-) than males. Additionally, the numbers of this cell population fluctuate significantly when female mice are infected at different points in their cycle, with mice infected during metestrus having higher proportion of microglia compared to mice infected during estrus or proestrus, further indicating a role of female gonadal hormones in the immune response. Furthermore, we assessed differences between males and females in expression of various genes known to be involved in the antiviral response in monocytes/microglia. Interestingly, we found sex differences in the expression of Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (APOBEC1) and its cofactor RNA binding motif protein 47 (RBM47), factors which have previously been shown to be involved in the attenuation of viral-induced encephalitis. At steady state, both males and females show similar expression levels of both APOBEC1 and RBM47 in the olfactory bulb. At four days post infection, however, females show significantly higher expression levels of both factors than both infected males and steady state females. We will discuss the role of RNA editing enzyme APOBEC1 in microglia in the sexually dimorphic response to VSV infection

Disclosures: K. Hajdarovic: None. **K. Gagnidze:** None. **D.C. Cole:** None. **K. Bulloch:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.14/Z12

Topic: E.02. Neuroimmunology

Support: Alberta Innovates Health Solutions - Graduate Studentship (PhD).

Canadian Institutes of Health Research (CIHR)

Title: Gestational bisphenol-A exposure increases susceptibility of adult mice to development of EAE through the innate immune system

Authors: *J. ROGERS[1], M. MISHRA[1], C. SILVA[1], O. KOVALCUK[2], L. METZ[1], V. YONG[1]; [1]Univ. of Calgary - Hotchkiss Brain Inst., Calgary, AB, Canada; [2]Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Multiple Sclerosis (MS) is a debilitating disease of the central nervous system (CNS) with pathology that includes inflammatory lesions, demyelination and axonal loss. This damage in caused by inflammatory lymphocytes and monocytes crossing the blood-brain barrier into the CNS parenchyma. Several lines of evidence suggest that environmental factors play an important role in shaping immune responses including the immune system of MS patients. Bisphenol-A (BPA) is a persistent environmental contaminant and an endocrine disrupting chemical. Studies have shown that BPA can be detected in up to 90% of human serum samples in various regions of the world. This suggests significant and widespread human exposure. Furthermore, it has been

shown that BPA can act on multiple systems and derail homeostasis. Specifically, studies have shown that during early life animals are more susceptible to lasting effects of BPA. To investigate a possible role for BPA in MS we used the Experimental Autoimmune Encephalomyelitis (EAE) model in C57BL/6 mice. During gestation pregnant mice were treated daily with either BPA or vehicle. Pups were then allowed to grow for 8-10 weeks undisturbed. EAE was induced using myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant without the use of pertussis toxin (PTx). In agreement with incidence reported in the literature, few control mice (no gestational exposure) or gestationally-exposed vehicle mice developed EAE when PTx was not used in the disease-inducing immunization procedure; in contrast, males who had been gestationally-exposed to BPA showed a significant increase in disease incidence. Examination of blood from gestationally-exposed mice when these were young adults showed a significant change in miRNA profile that is linked in the literature to dysregulation of the innate immune system. Moreover macrophages from gestational BPA-exposed mice appeared to have greater propensity to be activated in culture along a pro-inflammatory pathway. These results suggest that animals gestationally exposed to BPA may have increased susceptibility to EAE in adulthood through dysregulation of innate immunity. Gestational exposure to environmental factors that alter the immune system in later life may constitute a mechanism that predisposes an individual to MS.

Disclosures: J. Rogers: None. **M. Mishra:** None. **C. Silva:** None. **O. Kovalcuk:** None. **L. Metz:** None. **V. Yong:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.15/Z13

Topic: E.02. Neuroimmunology

Support: NIH Grant R01-MH082900

Pre-doctoral Bridge Funding from UNTHSC

Title: 17Beta-estradiol (E2) suppresses neuronal cyclooxygenase 2 gene expression

Authors: *W. STACEY[1], R. M. UHT[1,2];
[1]Pharmacol. and Neurosci., [2]Inst. for Aging and Alzheimer's Dis. Res., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Histological analyses of postmortem human brains suggest that neuronal cyclooxygenase-2 (COX-2) is upregulated in early stages of Alzheimer's disease (AD) (Ho, 2001). COX-2 is selectively expressed in a subset of neurons in the hippocampus, cerebral cortex, and amygdala (Yamagata, 1993). Although the suppressive effects of E2 on

cyclooxygenase-2 gene (cox-2) expression in the periphery are known, E2 effects on neuronal cox-2 remain uncharacterized. Data indicate that E2 and ERβ agonist Diarylpropionitrile (DPN) suppress COX-2 pre-mRNA and mRNA levels to the same extent. Furthermore, PHTPP a selective ERβ antagonist reversed the effect of both E2 and DPN. Because the cox-2 promoter lacks palindromic estrogen response elements, we targeted a proximal promoter region with a NF-κB response element implicated in cox-2 regulation. Using chromatin immunoprecipitation (ChIP), we analyzed changes with respect to promoter occupancy in the cox-2 proximal promoter region in response to E2 or DPN. Both ligands decreased NF-κB-p65 occupancy. Given that histone deacetylases (HDACs) may inhibit NF-κB activity, we sought to determine whether or not E2 repression of NF-κB-p65 occupancy involves HDACs. Treatment with the HDAC inhibitor trichostatin A (TSA) abrogated E2 and DPN suppression of COX-2 pre-mRNA. In keeping with the effect of TSA, E2 and DPN increased HDAC1 promoter occupancy; however recruitment of HDAC3 was unchanged. HDAC1 is known to form a complex with Sin3A, and E2 and DPN increased Sin3A occupancy. Interestingly, E2 and DPN both increased CBP occupancy. The recruitment of HDAC1 seems to correlate with decreased acetylation of histone 4 and not histone 3. Taken together, these data suggest that E2 suppresses neuronal cox-2 expression through ERβ-mediated recruitment of HDAC1 and Sin3A and a concomitant reduction of H4 acetylation. The role of CBP in this mechanism will be discussed.

Disclosures: W. Stacey: None. **R.M. Uht:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.16/Z14

Topic: E.02. Neuroimmunology

Title: Acute onset inflammatory demyelinating polyradiculoneuritis in an infant affected by severe combined immunodeficiency (SCID) due to RAG-1 hypomorfic mutation

Authors: *A. LOGOTETA[1], S. GALASSI[2], S. PRO[3], M. DI CAPUA[3], R. GRADINI[4], B. BERNARDI[2], D. LONGO[2];
[1]Dip. di Neuroscienze, Facoltà di Medicina e Chirurgia, Univ. La Sapienza, Azienda Ospedaliera Sant'Andrea, U.O.C. Neurologia, Roma, Italy; [2]Dept. Immagini, Ospedale Pediatrico Bambino Gesù, IRCCS, U.O. Neuroradiologia, Roma, Italy; [3]Dept. di Neuroscienze e Neuroriabilitazione, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy; [4]Dept. di Medicina Sperimentale, Univ. di Roma "La Sapienza", Roma, Italy

Abstract: An infant of 11 months suffering from SCID and polyradiculoneuritis was followed for two years from June 2012 to May 2013. Five days after the onset of clinical symptoms, the patient showed absence of reflexes, weakness of the limbs and bilateral ptosis. Lab. Data showed

hypergammaglobulinemia, CD4 + penia, PCR blood with CMV +, absence of both anti GQ1B-1 Ab and GM1- IgG/IgM. CSF examination showed increased proteins and IgG oligoclonal bands. Genetic analysis showed hypomorphic mutations of the RAG-1 gene. Electroneurography (ENG) was normal. MRI showed enhancement of roots of the cauda equina, III, IV, and VI cranial nerves and optic chiasm. IVIG treatment (2gr/kg in five days) + methylprednisolone was started. ENG at day 10 showed a demyelinating polyneuropathy. The patient continued the treatment with IVIG + methylprednisolone + ganciclovir. At day 21 the patient showed clinical worsening with ophthalmoplegia and respiratory failure and underwent plasmapheresis (8 cycles) combined with anti-CD20 (4 cycles) treatment. This therapy led to a mild symptomatological improvement. A further ENG evaluation at 70 day confirmed F-wave chronodispersion. On February 2013, MRI analysis showed unmodified neuroradiological lesions but the patient was still presenting a severe disability and underwent allogenic hematopoietic stem cells transplantation. After the transplantation the patient showed fair resistance to forced eyelid opening, good control of the head and trunk, spontaneous movements of both the upper and lower limbs with antigravitary contractions. Given the variety of symptoms, it was not easy to link the clinical data to the genetic mutation. MRI analysis was essential in the early stages of the disease to guide the diagnosis. This case is to our knowledge the first patient with a phenotype characterized by RAG-1 deficiency SCID-related polyradiculoneuritis.

Disclosures: **A. Logoteta:** None. **S. Galassi:** None. **S. Pro:** None. **M. Di Capua:** None. **R. Gradini:** None. **B. Bernardi:** None. **D. Longo:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.17/Z15

Topic: E.02. Neuroimmunology

Support: NIH Grant 1F31 MH102070-01A1

NIH Grant R01 MH090127

Title: Brain region specificity of inflammation-associated kynurenine metabolism

Authors: ***J. M. PARROTT**, L. REDUS, A. M. DUGAN, J. C. O'CONNOR;
Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Clinical and preclinical studies have established an association between the kynurenine pathway of tryptophan metabolism and the development of depression symptoms or depressive-like behaviors. During neuroinflammation, kynurenine metabolism is up-regulated and results in the production of neurotoxic metabolites which are hypothesized to contribute to depressive-like behaviors. Though recent analysis characterized the specific changes of whole

brain kynurenine metabolites in response to peripheral immune challenge, it is unknown if this metabolic response exhibits any regional specificity. Symptoms of depression and distinct depressive-like behaviors can be facilitated by different brain circuitry. Therefore, regional characterization of kynurenine metabolism might allow for better understanding of the potential mechanisms that mediate inflammation-associated behavior changes. To directly address this gap in understanding, four microdissected brain regions relevant to depression-related symptoms (dorsal and ventral hippocampus, central nucleus of the amygdala, nucleus accumbens) were analyzed. Kynurenine metabolites were measured by LC/MS 24h after peripheral (i.p.) administration of either lipopolysaccharide (LPS, 0.5mg/kg) or saline. In all regions assessed, LPS administration resulted in an increase in kynurenine and the kynurenine/tryptophan ratio. Interestingly, downstream metabolism following LPS injections varied between the four regions assessed. Neurotoxic 3-hydroxykynurenine was elevated in all regions except the ventral hippocampus and 3-hydroxyanthranillic acid increased in the dorsal hippocampus but was decreased in the nucleus accumbens. Kynurenic acid and xanthurenic acid, both previously demonstrated to not respond to neuroinflammation, was increased in the nucleus accumbens. Xanthurenic acid was also elevated in both the dorsal and ventral hippocampus. Together these data suggest inflammation-induced changes in kynurenine metabolism are brain region specific which has therapeutic implications for a number of neuropsychiatric disease populations.

Disclosures: J.M. Parrott: None. **L. Redus:** None. **A.M. Dugan:** None. **J.C. O'Connor:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.18/Z16

Topic: E.02. Neuroimmunology

Support: Polish National Science Center grant no. UMO-2012/07/B/NZ4/00205

Title: Subdiaphragmatic vagotomy does not protect against the increase of cerebral noradrenergic response in rat

Authors: *M. WIECZOREK[1,2], A. KOBRZYCKA[3], A. H. SWIERGIEL[4], E. OCLON[5], J. ZUBEL[5], M. HUBNER[3], M. SIUDAK[4];
[1]Univ. of Lodz, Fac. of Biolgy and Environm, Lodz, Poland; [2]Dept. of Neurobio., Univ. of Lodz, Lodz, Poland; [3]Neurobio., Univ. of Lodz, Fac. of Biol. and Envrn. Protection, Lodz, Poland; [4]Biol., Univ. of Gdansk, Gdansk, Poland; [5]Animals Physiol. and Endocrinol., Agr. Univ., Krakow, Poland

Abstract: Peripheral administration of gram-negative bacteria endotoxin - lipopolisaccharide (LPS) is known to activate the hypothalamo-pituitary adrenal axis (HPAA) and brain noradrenergic systems. We studied responses to peripherally administered LPS using the HPLC-ED to measure the concentration of noradrenaline and its metabolite MHPG in various brain regions of vagotomized rats. Rats were submitted to subdiaphragmatic vagotomy and after 30 days were used for experiments. They were injected with saline and LPS (10 μg ip) in random order, and two hours after the injections they were euthanized. The brains where removed from the skull and the hypothalamus, amygdala, prefrontal medial cortex, hippocampus, periaqueductal gray matter, and the brainstem were isolated. HPLC analysis indicated that subdiaphragmatic vagotomy did not protect against increase of noradrenaline concentration in analyzed brain regions. In case of LPS injected control animals we observed increased noradrenaline concentration versus saline injected ones. These results were comparable with those observed in sham operated rats. There results suggest that there may be compensatory mechanisms responsible for transferring of immune signal to the brain that develop during a relatively long time of recovery after subdiaphragmatic vagotomy.

Disclosures: **M. Wieczorek:** None. **A. Kobrzycka:** None. **A.H. Swiergiel:** None. **E. Oclon:** None. **J. Zubel:** None. **M. Hubner:** None. **M. Siudak:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.19/Z17

Topic: E.02. Neuroimmunology

Support: Philanthropic support from Don and Fran Herdrich

Title: *In vivo* measurement of retinal ganglion cell health following optic nerve injury or demyelination in preclinical models of multiple sclerosis

Authors: *K. MIRCHIA[1], C. HOWE[2];
[1]Neurol., [2]Mayo Clin., Rochester, MN

Abstract: Optic neuritis (ON) is the leading cause of disability in multiple sclerosis and neuromyelitis optica. Clinically, retinal nerve fiber layer (RNFL) thickness assessed via optical coherence tomography (OCT) is used as a diagnostic tool for optic neuritis. However, diagnostic potential in early disease is limited due to acute RNFL swelling. Recent reports indicate that retinal ganglion cell (RGC) loss precedes thinning of the RNFL, suggesting that sensitive measurement of the ganglion cell layer alone or in conjunction with the RNFL may provide earlier diagnostic utility. However, the mechanisms involved in RGC dropout during ON are not well established and detailed relative kinetics are lacking. Therefore, we sought to provide a

more detailed characterization of acute RGC responses in experimental models of ON. The unique transparency of ocular tissues coupled with the multitude of available intraoptic imaging techniques allows for robust and efficient *in vivo* analysis of neuronal responses to inflammation and demyelination. Using both the cuprizone model of chemically-induced optic nerve demyelination and the experimental autoimmune encephalomyelitis model of neuroinflammatory demyelination, we report *in vivo* measurements of pathophysiological changes in retinal tissue. Specifically, we have combined conventional bright-field fundus imaging and OCT with fluorescent imaging to synchronously study how optic nerve demyelination contributes to: 1) RGC dropout and morphological changes, using an adeno-associated viral vector to drive eGFP expression in neurons; 2) alterations in retinal vasculature, using intravenous delivery of TRITC-labeled dextran; and 3) retrograde axonal transport defects in the optic nerve following stereotactic delivery of Fluorogold tracer to the superior colliculus. These findings will inform future studies investigating the role of T cell-mediated injury of optic nerve axons during chemical or neuroinflammatory demyelination.

Disclosures: K. Mirchia: None. **C. Howe:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.20/Z18

Topic: E.02. Neuroimmunology

Support: Z01ES101623

Z01ES021164

Title: Arsenic exposure downregulates LPS or IL-4/IL-13 induced M1/M2 immune factors

Authors: *G. J. HARRY, R. ORIHUELA, C. A. MCPHERSON;
Natl. Toxicology Program Lab., Natl. Inst. Enviro Hlth. Sci., Durham, NC

Abstract: One primary health effect of inorganic arsenic (iAs) exposure relates to altered immune system function often resulting in a decreased ability of the system to mount an adequate response to infection, decreased T cell activation, and macrophage phagocytosis. Effects of arsenic exposure on the developing brain involve aspects of neuronal circuitry and connectivity. Given the link between microglia and synapses we examined the potential immune-dysregulatory effects of iAs exposure on microglia as a potential underlying mechanism for neurological effects. In BV-2 microglia cells, 24 hr exposure to iAs (1uM) decreased basal IL-4 and IL-1beta mRNA levels yet increased levels of LPS induced IL-1b. At 2.5 uM iAs, LPS induction of IL-1alpha, IL-1beta, IL-6, and iNOS mRNA levels was blunted. By 3 weeks of exposure, basal mRNA levels for M1 and M2 related cytokines were slightly elevated and levels

following LPS or IL4/IL13 induction significantly blunted. In primary microglia, 1uM iAs lowered basal levels of IL-1beta, IL-6, Arg-1 and IL-1RA mRNA and elevated LPS induction of IL-6. CD1 male mice exposed to 42.5 ppm iAs in the drinking water for 6 weeks produced comparable arsenic levels in the brain as those used *in vitro*. Under these conditions, iAs did not alter basal levels of M1 or M2 cytokines however, responses to LPS or IL4/IL13 were significantly blunted. Phagocytic actions of BV-2 cells exposed to arsenic did not appear to be compromised as measured by fluorescent bead-uptake and Image-Stream flow cytometry however, with increasing demand cells displayed a diminished level of bead uptake. Our data suggests that chronic exposure to arsenic significantly alters the brain immune system with a blunting of the normal protective response to challenge. Such alterations can have implications for brain development and for repair capabilities and plasticity later in life.

Disclosures: G.J. Harry: None. **R. Orihuela:** None. **C.A. McPherson:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.21/Z19

Topic: E.02. Neuroimmunology

Support: NIH grant RO1 MH083767

NIH grant RO1 MH101145

NIH grant R01 SUB UT 00000712

Title: Corticosteroids promote indoleamine/tryptophan-2,3-dioxygenase expression concomitantly with suppressing pro-inflammatory cytokines in the mouse brain

Authors: A. KELLY[1], M. A. LAWSON[2], K. W. KELLEY[2], *R. H. MCCUSKER[2];
[1]Neurosci. Program, [2]Animal Sci., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: An association between an activated immune system, sickness behaviors, and major depressive disorder (MDD) is well established. Frequently, MDD is associated with elevated corticosteroid levels and an elevation in tryptophan metabolism towards the production of kynurenine via the action of indoleamine/tryptophan-2,3-dioxygenase's (DO's: Ido1, Ido2 and Tdo2). A direct or interactive role of elevated corticosteroids on cytokine and DO expression within the brain relative to sickness or MDD is poorly defined. Here, we investigated the interaction between corticosteroid receptor agonists and inflammatory signals within the brain using an *ex vivo* model to quantify changes in pro-inflammatory cytokine and DO mRNA expression. Organotypic hippocampal slice cultures were prepared from 7-9 day old C57BL/6J mice and treated on day 7 under serum-free conditions with either dexamethasone (Dex, a

glucocorticoid receptor agonist) or aldosterone (Aldo, a mineralocorticoid receptor agonist) with or without pro-inflammatory mediators (LPS, poly I:C, IFNγ, IFNα). Both LPS and poly I:C increased expression of pro-inflammatory cytokines, TNFα and IL-6. This inflammatory response was attenuated in the presence of Dex, but not Aldo, which characterizes a prototypical anti-inflammatory glucocorticoid receptor-mediated response. Dex and Aldo had no effect on Ido1 expression when added alone. Only IFNγ induced the expression of Ido1, but surprisingly both Dex and Aldo interacted with IFNγ to accentuate expression of specific Ido1 transcripts. Ido2 expression was increased by LPS, poly I:C and IFNγ. Aldo also induced expression of Ido2 transcripts, both alone and in the presence of inflammatory mediators, while Dex had no effect. Tdo2 expression was increased only by Dex, a response unaltered by inflammatory mediators and not mimicked by Aldo. These results advance an emerging hypothesis that corticosteroids play a dichotomous role in the brain via interaction with the central innate immune system. In one role, glucocorticoids elicit an anti-inflammatory response, an action well established to be related to sickness recovery. In another role, corticosteroids may act through glucocorticoid and mineralocorticoid receptors to differentially enhance the expression of Ido1, Ido2, and Tdo2 within the brain. It is now necessary to characterize these newly discovered interactions *in vivo* to define the mechanism by which pro-inflammatory cytokines and stress hormones synergize relative to MDD.

Disclosures: A. Kelly: None. **M.A. Lawson:** None. **K.W. Kelley:** None. **R.H. McCusker:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.22/Z20

Topic: E.02. Neuroimmunology

Title: Participation of the inflammatory process in the treatment of patients with extraparenchymal neurocysticercosis

Authors: *Y. MARTINEZ LOPEZ, O. HERRERA VAZQUEZ, H. JUNG, R. CARRILLO, E. GARCÍA, L. ADALID, A. TOLEDO, À. FLEURY;
Inst. Nacional De Neurología Y Neurocirugía, Mexico, Mexico

Abstract: Neurocysticercosis (NCC) is the most common helminth infection of the central nervous system; remains endemic in developing countries. The extraparenchymal forms continue presenting high morbidity, because a 30-40 % of patients do not respond to cysticidal treatment. Objective: Evaluate the relationship between demographic, immunological and response to treatment in patients with cysticidal extraparenchymal neurocysticercosis. Material and Methods: Prospective, longitudinal, comparative and analytical study. Patients with definitive diagnosis of

vesicular extraparenchymal neurocysticercosis were included . Lymphoproliferation assays were performed on peripheral blood pre and post treatment (4-6 months) using antigens of Taenia solium. Clinically and radiologically patients were evaluated before and after treatment, determining the response to it. One sample was taken for measurement of plasma albendazole sulfoxide at the end of treatment Results: The study included 20 patients (7 women, 13 men) with a mean age of 45 ± 10 years; 17 of them had multiple parasites and 3 only one. It was found that 6 patients did not respond to treatment, in two cases were found association with gender or the number of parasites (P = 0.2, P = 0.24), but no association with age (P = 0.003) was found. The most common symptoms were headache and intracranial hypertension in 75% (15/20). The intensity of the pre and postreatment specific lymphoproliferative response were similar in patients with and without response to treatment (P = 0.70). Patients with response to treatment had a higher increase in cerebrospinal fluid cellularity evaluated before treatment (P = 0.02). Discussion:The lack of response to treatment in patients with extraparenchymal neurocysticercosis is currently one of the biggest problem in the management of patients. Preliminary results of this study show that intensity of the inflammatory reaction is associated with a better response to treatment. Also results show that lymphocyte proliferation index could predict clinical and radiological outcome in patients with extraparenchymal neurocysticercosis. This results, which will be completed with the measurement of inflammatory proteins, are of interest because it could indicate that the use of corticosteroids to prevent inflammatory complications should be done individually by patients.

Disclosures: **Y. Martinez lopez:** None. **O. Herrera vazquez:** None. **H. Jung:** None. **R. Carrillo:** None. **E. García:** None. **L. Adalid:** None. **A. Toledo:** None. **À. Fleury:** None.

Poster

346. Neuroimmunology: Regulating Systems

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 346.23/Z21

Topic: E.02. Neuroimmunology

Support: NIH AG016710

Title: Primary and BV-2 murine microglia can be pharmacologically manipulated by epigenetic modifiers to mimic microglial senescence

Authors: ***S. MATT**, R. W. JOHNSON;
Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: The progressive decline in maintaining homeostatic function during aging can lead to genomic instability and cellular senescence that are primary risk factors for age-related pathologies such as autoimmune disorders and neurodegenerative diseases. This aging phenotype

can partially be explained by the imbalance between pro-inflammatory and anti-inflammatory states. Microglia, the brain's resident immune cells, play a major role in the age-associated shift of the inflammatory profile of the central nervous system (CNS). During aging, microglia develop a more inflammatory phenotype and have increased gene and protein expression of pro-inflammatory cytokines such as interleukin (IL)-1β. Further, when the immune system is challenged with a pro-inflammatory agent such as lipopolysaccharide (LPS), microglial activation is amplified and prolonged in the aged brain compared with adults, leading to exaggerated neuroinflammation, sickness behavior, and cognitive deficits. What causes these impairments and brings about a "primed" microglial population remains to be elucidated. Significant associations between aging and epigenetic alterations have recently been identified, notably that global DNA methylation decreases with age leading to a loss of phenotypic plasticity. We have previously demonstrated that aging-induced exaggerated pro-inflammatory cytokine gene expression in microglia was associated with DNA hypomethylation of the Il1b promoter, so we sought to determine whether adult primary microglia or the microglial BV-2 cell line could be modulated by epigenetic drugs similar to senescent microglia. We investigated whether the demethylating agent 5-azacytidine (5-aza) increased mRNA expression and decreased methylation of Il1b. Novel findings indicate that both 5-aza and/or LPS altered Il1b mRNA expression and DNA methylation of BV-2 and primary microglia as well as mRNA expression of epigenetic regulators including histone deacetylases and DNA methyltransferases. Manipulating the epigenetic landscape has enormous potential for improving our understanding of age-related neuroinflammatory complications and the role of microglial hyperactivity in the aged brain.

Disclosures: S. Matt: None. **R.W. Johnson:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.01/Z22

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR

AIHS

Petro-Canada Young Innovator Award

Title: Dynamic changes in dendritic spine numbers in an animal model of Multiple Sclerosis

Authors: *K. MILLOY[1], M. VERBEEK[1], S. ACHARJEE[2,1], Q. J. PITTMAN[2], A. BENEDIKTSSON[1];

[1]Mount Royal Univ., Calgary, AB, Canada; [2]Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Multiple sclerosis (MS), a demyelinating disease with sensory and motor deficits, is also associated with behavioural co-morbidities such as neuropsychiatric and cognitive impairments, affecting around 50% of patients. The mechanisms underlying these changes are poorly understood. However, inflammation occurring even in the early stages of the disease can alter brain structure and function, leading to these abnormalities. We investigated if there were any changes in dendritic spines using in an animal model of MS, called experimental autoimmune encephalomyelitis (EAE). We focused on the amygdala and hippocampus, brain regions involved in emotion processing and memory, respectively. Methodology: EAE was induced in C57/BL6 mice with MOG33-55/CFA and pertussis toxin (PTX). Control mice received only CFA and PTX. Neurons were labeled using Golgi staining at two timepoints: day (d7), when behavioral changes can be seen, and at d17, where motor deficits are evident. Neurons were imaged with confocal microscopy and analyzed using Imaris software to examine dendritic spines, which form the postsynaptic structure of the synapse. A minimum of 34 neurons was analyzed in each condition. Results: Spine number was increased by 22% ($p<0.05$) in the principal neurons of the basolateral amygdala at d7, a stage when the animals do not show any sign of paralysis but have increased levels of cytokines in the brain. At d17, the difference in the spine count between the EAE and controls disappeared, indicating there was most likely increased spine loss in EAE. We did not observe any change in the spine density in the hippocampus at d7 or 17. Conclusion: There appears to be a temporal and region specific alteration in the spine density in the brains of EAE mice. Further functional analysis, with electrophysiology, and further characterization of spines will shed light on how spine density influences synaptic function and circuit dynamics that lead to behavioural changes.

Disclosures: K. Milloy: None. **M. Verbeek:** None. **S. Acharjee:** None. **Q.J. Pittman:** None. **A. Benediktsson:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.02/Z23

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSF-DOD grant #PHY 1004860 (REU program)

Title: Suppression of microglial activation with PPAR-gamma agonists and anti-histamines: relevance to multiple sclerosis

Authors: *P. D. STORER, K. E. ROHR, C. S. MILLER, B. L. WADSWORTH;
Biol., Coe Col., Cedar Rapids, IA

Abstract: Multiple sclerosis (MS) is a chronic, progressive disease involving immunological damage to myelin sheaths and nerve cells in the brain and spinal cord. Microglial cells are thought to play a key role in the immune response of the central nervous system and are thus implicated in the pathology of MS. Once activated, microglia function to eliminate pathogens and secrete cytokines to promote further pro-inflammatory responses. However, chronically activated microglia fuel a self-renewing cycle of activation that results in the observed oligodendrocyte and neuron damage. In this study, a microglial cell line (N9) was used to study the potential anti-inflammatory properties of H1R-specific anti-histamine compounds following immunologically-relevant activation. The rationale for these studies is based on the clinical relevance of anti-histamines and their ability to control various aspects of the immune system in other disease models. Experimental comparisons were made to the anti-inflammatory properties of other known compounds including the PPAR-gamma agonists rosiglitazone and 15d-PGJ2. Results from epPCR, qPCR, ELISA and IHC studies confirmed the ability of PPAR-gamma agonists and demonstrated the ability of anti-histamines, namely clemastine, to attenuate nitric oxide production and cytokine/chemokine expression in N9 cells. These results suggest a potentially new combinatorial avenue at controlling inflammation in the CNS by targeting the inflammatory mechanisms of microglia cells.

Disclosures: P.D. Storer: None. **K.E. Rohr:** None. **C.S. Miller:** None. **B.L. Wadsworth:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.03/Z24

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Homeostatic role of neuronal L1 cell adhesion molecule during inflammatory processes in the central nervous system

Authors: *L. MENZEL[1], M. PATERKA[2], R. WHITE[3], M. SCHACHNER[5], F. ZIPP[2], M. SCHÄFER[4];
[2]Neurol., [3]Inst. of Physiol., [4]Anesthesiol., [1]Univ. Med. Ctr. Mainz, Mainz, Germany; [5]Ctr. for Mol. Neurobio., Hamburg, Germany

Abstract: Neuroinflammatory diseases such as multiple sclerosis (MS) are associated with infiltration of peripheral immune cells into the CNS. It remains unclear how the CNS adapts to the presence of peripheral immune cells and autoimmune-directed inflammatory attacks.

Sustained contacts between immune cells and neurons have been reported in Experimental Autoimmune Encephalomyelitis (EAE), a rodent model of MS. This finding implies the existence of molecules mediating cell adhesion and recognition as well as reciprocal signaling between neurons and immune cells. In this study, we investigated a role of the neuronal L1 cell adhesion molecule (L1CAM) in neuroinflammation using EAE in mice as a model. L1CAM protein expression in spinal cord was downregulated at the peak of disease. In order to examine the relevance of reduced neuronal L1CAM expression in EAE, neuron-specific L1CAM knockout mice were analyzed. Severity progression of EAE was significantly attenuated in these mice compared to control. *In vitro* experiments further demonstrated a rapid downregulation of neuronal L1CAM mRNA expression in primary neurons when co-cultured with activated $CD4^+$ T cells. These results suggests that neuronal downregulation of L1CAM is triggered by T cell signaling and thereby ameliorates neuroinflammatory disease in a preclinical model of MS. Our results further support a role of L1CAM for CNS homeostasis in response to inflammatory processes.

Disclosures: **L. Menzel:** None. **M. Paterka:** None. **R. White:** None. **M. Schachner:** None. **F. Zipp:** None. **M. Schäfer:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.04/Z25

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Marie Curie ITN nEUROinflammation FP7

Title: Early biomarkers for demyelination and neuroinflammation in the Experimental Allergic Encephalomyelitis (EAE), an animal model for Multiple Sclerosis

Authors: ***N. BORJINI**[1,2,4], M. FERNANDEZ[2], S. SIVILIA[3], L. GIARDINO[4,3], L. CALZÀ[4,2]; [1]Chiesi Farmaceutici Spa, Parma, Italy; [2]Hlth. Sci. and Technologies Interdepartmental Ctr. for Industrial Res. (HST-ICIR), [3]Dept. of Vet. Med. Sci., Univ. of Bologna, Ozzano Emilia (BO), Italy; [4]IRET Fndn., Ozzano Emilia (BO), Italy

Abstract: Inflammation and demyelination are the primary pathologies in multiple sclerosis (MS) lesions and in the EAE (experimental allergic encephalomyelitis), a widely used experimental animal model for this human disease. Despite the prevailing efforts made in the field of biomarkers to determine prognostic factors and predict the clinical disease course, a correlation between tissue neuroinflammation, demyelination, neurodegeneration and potential biomarkers in biological fluids remain to be defined. In this study, we perform a time-course investigation based on a discovery strategy of myelination and inflammation biomarkers in

cerebrospinal fluid (CSF) and spinal cord (SC), using high-throughput technologies in order to highlight the potential of novel early biomarkers for MS. Female Dark-Agouti rats were immunized using an emulsion of guinea pig spinal cord, heat-inactivated Mycobacterium tuberculosis and complete Freund's adjuvant and were daily weighed and examined for clinical score. At 1, 5, 8, 11 and 18 day post-immunization (DPI), EAE rats were sacrificed, CSF was collected and total SC was dissected. We performed multiparametric quantification of inflammatory mediators in the CSF through xMAP technology and Luminex platform. We used the SC for investigating inflammation and myelination markers by real-time PCR array and immunohistochemistry. Several proteins significantly changed in the CSF of EAE animals compared with control. In particular, the pro-inflammatory cytokines/chemokines IL1b, CCL5, TNFa and the anti-inflammatory such as IL5, IL10, VEGFA are upregulated starting from 8 DPI. Regarding gene expression, myelination genes were highly down-regulated starting from 5 DPI, especially MAL, MBP, PMP22 while an opposite expression profile was observed for inflammation related genes, for instance CXCL11, CXCL9 and LTA were the most upregulated genes showing 100 fold change at 8 DPI, around 300 at 11 DPI and 125 at 18 DPI. SC inflammatory infiltrates were identified in EAE animals and double staining with antibodies markers of microglia and myeloid (CD11b) M1 (CD86), M2 (CD163) phenotypes, T cells (CD44), astroglia (GFAP) oligodendrocyte precursor cells at different maturation stages (PDGFaR, NG2, CNPase, MBP) and neurons (b-tubulin, NSE) was performed in order to identify the producing cell type. This early biomarkers regulation reflects the immunological response and the demyelination process taking part as soon as 5 DPI, before the clinical onset of EAE. Most regulated genes/proteins will be processed through pathway databases in order to figure out possible mechanisms regulating inflammation/demyelination events during EAE.

Disclosures: **N. Borjini:** None. **M. Fernandez:** None. **S. Sivilia:** None. **L. Giardino:** None. **L. Calzà:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.05/Z26

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Italian Ministry of Health GR-2011-02347036

Title: Role of miR-142-3p in inflammation-dependent synaptic dysfunctions affecting a mouse model of multiple sclerosis

Authors: **F. DE VITO**[1,2], *G. MANDOLESI[1], A. MUSELLA[1], A. GENTILE[2,1], D. FRESEGNA[2,1], S. BULLITTA[1], H. SEPMAN[2], N. HAJI[1], C. DI SANZA[1], E. HORNSTEIN[3], I. BOZZONI[4], C. PRESUTTI[4], D. CENTONZE[1,2];

[1]IRCCS-Santa Lucia Fdtn, Rome, Italy; [2]Tor Vergata Univ. of Rome, Rome, Italy; [3]Weizmann Inst. of Sci., Rehovot, Israel; [4]Sapienza Univ. of Rome, Rome, Italy

Abstract: MicroRNAs (miRs) are small non-coding RNAs, which regulate several physiological and pathological processes by repressing post-transcriptionally target mRNAs. Recently, the involvement of miRs emerged also in multiple sclerosis (MS), a chronic inflammatory and degenerative disease of the central nervous system. Although their role is mainly related to the immune system, little is known about miRs in MS grey matter pathology, in particular in the inflammatory-dependent synaptopathy, which affects MS and its mouse model experimental autoimmune encephalomyelitis (EAE). Aims: We asked whether a miR dysregulation is implicated in the interleukin1-β (IL1-β) dependent synaptic alterations, which affect the EAE cerebellum, leading to excitotoxic damage. Methods: MOG(35-55)-EAE was induced in female C57BL/6 mice. miR expression was analyzed by microarray and qRT-PCR in EAE cerebellum and by qRT-PCR in cerebrospinal fluids (CSFs) of MS patients (gadolinum-positive, Gd+ vs gadolinum-negative, Gd-). The role of miR deregulation in EAE cerebellar dysfunction was explored by miR over-dosage or inhibition *in vitro* (report assays), *ex vivo* or *in vivo* (lentiviral approach, miR KO mice or LNA anti-miR by intracerebroventricular infusion, ICV), followed by immunofluorescence and electrophysiological analyses. Results: We identified miR-142-3p as the most increased miR in the EAE cerebellum during the acute phase of the disease and demonstrated that it down-regulates GLAST/EAAT1, a glial glutamate-aspartate transporter important for synaptic glutamate uptake and strongly compromised in EAE in an IL1-β-dependent way. In support of an IL1-β-miR-142-3p-GLAST regulatory axis, we showed that incubation of IL1-β on normal cerebellar slices induced miR-142-3p up-regulation, GLAST down-regulation and an enhancement of the glutamatergic transmission as in EAE. Of note, the IL1-β synaptic effect failed in miR-142 KO slices as well as a recovery of the glutamatergic transmission was observed in EAE miR-142-3p KO mice or in a group of EAE mice preventively treated by ICV infusion with a miR-142-3p inhibitor. Finally, we provide evidence of an involvement of miR-142-3p in MS disease not only by detecting elevated miR levels in the CSF of Gd+ MS patients but also by reproducing the EAE synaptic alterations in cerebellar slices incubated with the Gd+ CSF. Interestingly, pre-incubation of the Gd+ MS CSF with a miR-142-3p inhibitor was protective. Conclusions: Our findings highlight miR-142-3p as key molecular player in IL1-β-mediated synaptic dysfunction and excitotoxic damage in both EAE and MS pathophysiology, with potential therapeutic implications.

Disclosures: F. De Vito: None. **G. Mandolesi:** None. **A. Musella:** None. **A. Gentile:** None. **D. Fresegna:** None. **S. Bullitta:** None. **H. Sepman:** None. **N. Haji:** None. **C. Di Sanza:** None. **E. Hornstein:** None. **I. Bozzoni:** None. **C. Presutti:** None. **D. Centonze:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.06/Z27

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Recurrent herpes simplex virus-1 (HSV1) infections in mice cause signs of neurodegeneration and cognitive deficits

Authors: *G. DE CHIARA[1], M. FABIANI[2], D. LIMONGI[3,4], A. MASTRODONATO[5], R. PIACENTINI[5], M. E. MARCOCCI[2], P. COLUCCIO[2], C. GRASSI[5], A. T. PALAMARA[2,6,4]; [1]Inst. of Translational Pharmacol., Natl. Res. Council, Rome, Italy; [2]Dept. of Publ. Hlth. and Infectious Dis., Sapienza Univ. of Rome, Rome, Italy; [3]Telematic Univ. San Raffaele, Rome, Italy; [4]IRCCS San Raffaele Pisana, Rome, Italy; [5]Inst. of Human Physiol., Univ. Cattolica del Sacro Cuore, Rome, Italy; [6]Pasteur Institute–Fondazione Cenci-Bolognetti-Sapienza Univ. of Rome, Rome, Italy

Abstract: We previously demonstrated that herpes simplex virus-1 (HSV-1) infection in neurons causes marked changes in their excitability and intracellular Ca2+ content, together with the phosphorylation and amyloidogenic processing of amyloid precursor protein (APP), (De Chiara et al, 2010, Piacentini et al, 2011, Civitelli et al 2015). These data support the hypothesis that recurrent HSV-1 reactivations may contribute to neurodegeneration typical of Alzheimer's disease (AD), causing repeated cycles of viral infection into the brain. To further explore such an hypothesis, we established a murine model of recurrent HSV-1 infection, closely resembling those occurring in humans: 1 month old female BALB/c mice were inoculated via snout abrasion with a sublethal doses of HSV-1 (F strain, 1x10e6 plaque forming unit), or a mock solution as control. Viral reactivation was periodically induced by thermal stress. HSV-1 spreading to the brain, as well as AD-like neuropathological hallmarks were analyzed in mice during the course of aging. Following virus reactivations we found: 1) viral TK and ICP4 genes (markers of viral infection and active replication, respectively) in cortex and hippocampal tissues, indicating that HSV-1 is able to reach and actively replicate in those brain regions mostly affected during AD; 2) accumulation of β-amyloid peptides (Aβs) and other APP proteolytic fragments, together with altered tau phosphorylation and signs of neuroinflammation in hippocampus and cortex of aged animals; 3) significant impairments in mouse performance in the novel object recognition and Y Maze behavioral tests. Overall, these results strongly support the hypothesis that recurrent HSV-1 infections may contribute to neurodegeneration typical of AD.

Disclosures: G. De Chiara: None. **M. Fabiani:** None. **D. Limongi:** None. **A. Mastrodonato:** None. **R. Piacentini:** None. **M.E. Marcocci:** None. **P. Coluccio:** None. **C. Grassi:** None. **A.T. Palamara:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.07/Z28

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Herpes simplex virus type-1 infection promotes inflammation in mouse derived DRG explants and primary neuronal cell culture model

Authors: *H. SHARTHIYA[1], C. SENG[2], V. TIWARI[3], M. FORNARO[1];
[1]Anat., [2]Biomed. Sci., [3]Microbiology & Immunol., Midwestern Univ., Downers Grove, IL

Abstract: The hallmark of herpes simplex virus (HSV) infection is to establish lifelong latency in dorsal root ganglion (DRG) explants. A multi-step based interactions between HSV and cell surface receptor leads to the viral entry and cell-to-cell spread. Interestingly the major players involved in promoting viral entry and inflammation in sensory nervous system remains poorly understood. In this study we established a mouse derived dorsal root ganglion (DRG) explants and DRG-explants derived neuronal cell culture model in order to study the significance of HSV glycoprotein D (gD) interactions to 3-O sulfated heparan sulfate (3-OS HS) entry receptor and associated inflammation. Our results indicates that both DRG-explants and explant derived neuronal cells were susceptible to HSV-1 entry and spread as positive ONPG, ×-gal staining and plaque formation were recorded using reporter HSV-1-expressing reporter gene (β-galactosidase) and replication competent HSV-1 (KOS 804) virus. Using confocal imaging, we also provide a visual evidence for cell surface 3-O sulfated heparan sulfate (3-OS HS) expression followed by HSV-1 glycoprotein D (gD) interaction to 3-OS HS receptor during viral entry. Our screening for a panel of pro-inflammatory cytokines upon HSV-1 infection provided an interesting link between upregulated cytokines and heparan sulfate (HS) implicating potential role of HS and associated neuroinflammation. Taken together, our results highlight a novel approach to study gD-3-OS HS interactions in order to develop therapeutics HSV mediated neuroinflammation.

Disclosures: **H. Sharthiya:** None. **C. Seng:** None. **V. Tiwari:** None. **M. Fornaro:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.08/Z29

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-GM 093869

NIH RCMI-G12RR0305

MBRS-RISE R25GM061838

Title: Neurotrophins affect the number and distribution of two macrophage subtypes after optic nerve injury

Authors: *G. S. VEGA MELÉNDEZ[1,2], M. V. DUPREY-DIAZ[1,2], J. M. BLAGBURN[1], R. E. BLANCO[1,2];
[1]Inst. of Neurobio., Old San Juan, PR; [2]Anat. and Neurobio., Univ. of Puerto Rico, Sch. of Med., San Juan, PR

Abstract: We have shown that ciliary neurotrophic factor (CNTF) and fibroblast growth factor (FGF-2) have strong enhancing effects on axonal regeneration in the adult frog optic nerve after injury. In these nerves, bundles of regenerating axons are associated with astrocytes and macrophage-like cells. The objective of the present study is to characterize and identify these cells, and to determine the changes that occur in them after axotomy and CNTF or FGF-2 application. We performed optic nerve crush and applied either saline solution or neurotrophic factors to the nerve. We examined the optic nerves at 48h, one week, and two weeks after axotomy. Ultrastructure studies of the macrophages/microglia after injury and neurotrophic treatment were carried out with electron microscopy of the proximal, injury, and distal sites. Immunocytochemistry for various macrophage subtypes was performed and confocal images of the different regions were taken. Ultrastructural examination of the nerves showed a large number of macrophage-like cells at the lesion site, and distally in close proximity to regenerating axons in CNTF and FGF-2 treated nerves. Significantly fewer cells were present proximal to the lesion. The vast majority of cells were ED-1-positive in saline-, CNTF-, and FGF-2-treated nerves. The distribution of these cells was quantitatively analyzed with antibodies against specific macrophage subtypes. Both pro-inflammatory M1 macrophages (CD-86-positive) and anti-inflammatory M2 macrophages (arginase-positive) were identified at the injury and distal sites. A small but significant increase in CD-86-positive cells (M1 subtype) was observed at 48h and one week after CNTF and FGF-2 application, when compared to saline-treated animals. In saline-treated nerves arginase-positive cells (M2 subtype) were present at one and two weeks after axotomy; treatment of the nerves with CNTF and FGF-2 significantly increased the number of labeled cells. We are also studying the secretions of different cytokines relevant to the presence of these macrophage subtypes. In conclusion, the application of CNTF and FGF-2 affects the number and the distribution of macrophage subtypes after optic nerve injury and it may play an important role in the success of optic nerve regeneration.

Disclosures: G.S. Vega Meléndez: None. **M.V. Duprey-Diaz:** None. **J.M. Blagburn:** None. **R.E. Blanco:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.09/Z30

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Brimonidine suppresses loss of retinal neurons and visual function in a murine model of optic neuritis

Authors: *X. GUO, K. NAMEKATA, A. KIMURA, T. NORO, C. HARADA, T. HARADA; Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: Brimonidine (BMD) is a selective α2-adrenergic receptor agonist that is used clinically for the treatment of glaucoma, one of the leading causes of irreversible blindness that is characterized by progressive degeneration of optic nerves and retinal ganglion cells (RGCs). BMD lowers intraocular pressure, but recent evidence suggests that its therapeutic efficacy may also mediate through mechanisms independent of modulation of intraocular pressure (IOP). We show that BMD prevented retinal degeneration in excitatory amino-acid carrier 1 (EAAC1)-deficient mice, a murine model of normal tension glaucoma (NTG), probably by suppressing the phosphorylation of the N-methyl-D-aspartate receptor 2B (NR2B) subunit in RGCs and by stimulating the production of several neurotrophic factors that enhance RGC survival in Müller glial cells. Optic neuritis is inflammation of the optic nerve and is strongly associated with multiple sclerosis (MS), an inflammatory demyelinating syndrome of the central nervous system. It leads to RGC death and can cause severe vision loss. To further elucidate the neuroprotective role of BMD, we examined the effects of BMD on optic neuritis during experimental autoimmune encephalomyelitis (EAE), an animal model of MS. EAE was induced with MOG35-55 in female C57BL/6J mice and BMD eyedrops were applied daily. In the EAE retina, the number of RGCs was significantly decreased and this effect was suppressed with BMD treatment. Consistent with histological analyses, the visual impairment observed in EAE mice, as assessed by multifocal electroretinograms (mfERGs), an established noninvasive method for reliably measuring visual function, was inhibited with BMD treatment, indicating the functional significance of the neuroprotective effect of BMD. Furthermore, BMD increased the expression level of the basic fibroblast growth factor in the EAE retina, particularly in Müller glial cells and RGCs. Our findings suggest that topical administration of BMD may be available for RGC protection during optic neuritis, as well as for glaucoma.

Disclosures: X. Guo: None. **K. Namekata:** None. **A. Kimura:** None. **T. Noro:** None. **C. Harada:** None. **T. Harada:** None.

Poster

347. Neuroinflammation: Multiple Sclerosis and Related Models

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 347.10/Z31

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: An application of a novel method to study cerebrospinal fluid dynamics in rats

Authors: ***J. K. KARIMY**[1], K. T. KAHLE[2], D. B. KURLAND[1], V. GERZANICH[1], J. M. SIMARD[1];
[1]Neurosurg., Univ. of Maryland, Baltimore, Baltimore, MD; [2]Neurosurg., Boston's Children's Hosp., Boston, MA

Abstract: Cerebrospinal fluid (CSF) flow dynamics play critical roles in both the immature and adult brain, with implications for neurodevelopment and disease processes such as hydrocephalus and neurodegeneration. Intracerebroventricular hemorrhage (IVH) is a deleterious CNS injury that affects thousands of people each year and often results in the formation of hydrocephalus. Unchecked, hydrocephalus can result in increased intracranial pressure, resulting in motor deficits and even death. Historically, hydrocephalus following IVH has been attributed to impaired reabsorption of CSF due to blockages in arachnoid granulations, however the contribution of injury-induced overproduction of CSF by the choroid plexus epithelium has been overlooked. Available tracer dilution methods do not permit ongoing real-time determination of the rate of CSF formation and are not readily amenable to pharmacological interventions, thus accurate characterization of CSF dynamics following CNS injury has been difficult. We recently published a simple method that permits the real-time measurement of the rate of formation of CSF in laboratory rats *in vivo* under near-physiological conditions. We hypothesized that this technique would be useful to study CSF production in a rodent model of IVH. To evaluate this, autologous blood (50 µl) was infused into the right lateral ventricle 48 hours prior to CSF measurements. Our results show a striking 3-fold increase in the rate of CSF production following IVH compared to age matched controls, 1.5 µl/min versus 0.5 µl/min, respectively. Overproduction of CSF following IVH correlated strongly with ventriculomegaly, a hallmark of hydrocephalus. To our knowledge, this is the first direct evidence that CSF overproduction may contribute to hydrocephalus following IVH. This observation has important clinical implications.

Disclosures: **J.K. Karimy:** None. **K.T. Kahle:** None. **D.B. Kurland:** None. **V. Gerzanich:** None. **J.M. Simard:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.01/Z32

Topic: E.04. Autonomic Regulation

Support: CONACYT 554957 PACM

VIEP MORC-NAT 64

CONACYT 172023 CPR

Title: Anatomic relationship between the ovaries and the autonomic and sensory ganglia during the estrous cycle in rats

Authors: *P. A. CRUZ MARTÍNEZ[1], Y. CRUZ GOMEZ[2], C. PASTELIN[3], A. HANDAL[3], C. MORAN[3];
[1]Univ. Autonoma De Tlaxcala, Puebla, Mexico; [2]Univ. Autonoma de Tlaxcala, Tlaxcala, Mexico; [3]Benemerita Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: The ovaries innervation involves two nerves: the superior ovarian nerve (SON) and the ovarian plexus nerve (OPN), which connects the ovaries with the prevertebral [celiac ganglia (CG), superior mesenteric ganglion (SMG), suprarenal ganglion (SG)], paravertebral, sympathetic chain ganglia (SCG) and T12-L2 dorsal root ganglia (DRG). The aims of the present study were to localize the postganglionic neurons innervating the right and left ovaries, and describe the fluctuations of the neurons in the different ganglia during the estrous cycle. The study was conducted in virgin adult CII-ZV female rats. The estrous cycle was monitored by daily vaginal smears and only cyclic rats were used. The retrograde tracer true blue (TB) was injected into the right or left ovarian bursa. Four days later, the rats were transcardially perfused and the prevertebral, paravertebral and dorsal root ganglia (T12-L2) were collected and analyzed for positive true blue neurons. In the animals injected in the left ovarian bursa in the estrous, the ipsilateral CG had the higher number of positive true blue neurons (43±10 vs. SCG 24.7±7, SG 15.5±5 $p<0.05$). Moreover the CG in diestrus 1 had the lowest number of positive neurons (7.5±2.1). In the animals injected in the right ovarian bursa, the ipsilateral CG had the highest number of positive true blue neurons (50±8.0 vs. SCG 12±2, SG 15±2.8, SMG 10±2 $p<0.05$). The positive neurons in the ipsilateral sensory ganglia in the animals injected in the left ovarian bursa was higher during the estrous phase, meanwhile in animals injected in the right bursa the higher number of positive neurons was during proestrus day. We conclude that postganglionic neurons controlling the ovarian functions are located in several ganglia and most of the posganglionic neurons are in the CG and sensorial ganglia. We confirm the changes in the pathways that connect the ovaries to the nervous system during the estrous cycle of the rat and their asymmetry.

Disclosures: P.A. Cruz Martínez: None. **Y. Cruz Gomez:** None. **C. Pastelin:** None. **A. Handal:** None. **C. Moran:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.02/Z33

Topic: E.04. Autonomic Regulation

Support: CONACYT 225126 (MMG)

PAPIIT-UNAM IN206013-3 (MMG)

CONACYT 417840 (RL)

Title: Changes in bladder and urethral function: a model of bulbospongiosus nerve crush in female rabbit

Authors: *D. L. CORONA QUINTANILLA[1,3], C. ACOSTA-ORTEGA[2], N. RODRÍGUEZ[4], O. SÁNCHEZ-ZAYAS[3], R. LÓPEZ-JUÁREZ[5], F. CASTELÁN[3], M. MARTÍNEZ-GÓMEZ[6]; [2]Biología, [1]Univ. Autónoma de, Tlaxcala, Mexico; [3]Ctr. Tlaxcala de Biología de la Conducta, [4]Biología, [5]Doctorado en Ciencias Biológicas, Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; [6]Inst. de Investigaciones Biomédicas, Dept. de Biología Celular y Fisiología, México, Mexico

Abstract: Pelvic and perineal floor muscles are crucial for the function of the urogenital system in female mammals. In women during childbirth these muscles and its innervation can be compressed or overstretched, and can be cause urinary and sexual dysfunction. The pudendal and perineal nerves are vulnerable to trauma during delivery. We showed that in multiparous rabitts does uncoordinated pattern of activity of the pelvic (pubococcygeus) and perineal (bulboesponjoso,Bsm) muscles and urinary dysfunctions. To discriminate if these changes are caused by damage to the innervation, specifically the of Bsm, to evaluate changes in the bladder and urethral function in a model of bulbospongiosus nerve crush. For experiments virgin rabbits (12 + 2 months n = 5) anesthetized with urethane 20%, which was underwent simultaneous cystometrograms, urethral and electromyogram records before and after of the crushing bulbospongiosus nerve. The results suggest that the nerve crushing produced voiding dysfunctions and inefficiency of the bladder contraction, a significant increase of the residual volume and urethral resistance similar at multiparous rabbits. It is possible that during the childbirth the perineal nerves, specifically the bulbospongiosus nerve, were compressed and postpartum, the women would develop sexual and urinary dysfunction.

Disclosures: D.L. Corona Quintanilla: None. **C. Acosta-Ortega:** None. **N. Rodríguez:** None. **O. Sánchez-Zayas:** None. **R. López-Juárez:** None. **F. Castelán:** None. **M. Martínez-Gómez:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.03/Z34

Topic: E.04. Autonomic Regulation

Support: NIH R01 NS050514

Title: Modeling the spinal pudendo-vesical reflex for bladder control by pudendal afferent stimulation

Authors: *M. J. MCGEE[1], W. M. GRILL[1,2,3,4];
[1]Biomed. Engin., [2]Electrical and Computer Engin., Duke Univ., Durham, NC; [3]Neurobio., [4]Surgery, Duke Univ. Med. Ctr., Durham, NC

Abstract: Electrical stimulation of afferents in the pudendal nerve (PN) is a promising approach to restore continence and micturition following bladder dysfunction resulting from neurological disease or injury. Although the pudendo-vesical reflex and its physiological properties are well established, there is limited understanding of the underlying neural network mechanisms that mediate the reflexes governing the effects of PN stimulation on bladder function. The objective of this work was to develop a biophysically-motivated model of the neural network underlying the pudendo-vesical reflex. We implemented and validated a neural network architecture based on previous neuroanatomical and electrophysiological studies. Using synaptically-connected integrate and fire model neurons, we created a network model with realistic spiking behavior. Our model reproduced the hallmarks of the pudendo-vesical reflex: volume-dependence of stimulation-evoked bladder contractions and stimulation frequency-dependent bladder activation. Further, the model reproduced the effects of pudendal afferent stimulation frequency and stimulation pattern measured experimentally, and allowed for exploration of the mechanisms underlying the strong stimulation frequency-dependent effects. The model produced expected sacral parasympathetic nucleus (SPN) neuron firing rates from prescribed neural inputs and predicted bladder activation and inhibition with different frequencies of pudendal afferent stimulation. In particular, the model matched experimental results from previous studies of temporal patterns of pudendal afferent stimulation and selective pharmacological blockade of inhibitory neurons. Although the model included descending supraspinal control of the SPN, the frequency and pattern-dependent effects of pudendal afferent stimulation were determined by changes in firing rate of spinal interneurons, suggesting that neural network interactions at the sacral level can mediate the bladder response to different frequencies and temporal patterns of pudendal afferent stimulation. The anatomical structure of excitatory and inhibitory interneurons in the network model was necessary and sufficient to produce the critical features of the pudendo-vesical reflex and may prove useful to guide development of novel, more effective electrical stimulation techniques for bladder control.

Disclosures: M.J. McGee: None. **W.M. Grill:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.04/Z35

Topic: E.04. Autonomic Regulation

Support: GlaxoSmithKline

Title: Pelvic nerve stimulation restores bladder capacity and voiding efficiency in rat prostaglandin E2 overactive bladder model

Authors: *C. L. LANGDALE[1], J. A. HOKANSON[1], A. SRIDHAR[5], W. M. GRILL[1,2,3,4]; [1]Biomed. Engin., [2]Electrical and Computer Engin., [3]Neurobio., [4]Surgery, Duke Univ., Durham, NC; [5]Bioelectronics R&D, GlaxoSmithKline, Stevenage, United Kingdom

Abstract: Overactive bladder (OAB), resulting in urgency, frequency, and incontinence, is a highly prevalent condition that leads to medical complications and decreased quality of life. Treatments for OAB include behavioral therapy, exercise therapy, pharmacotherapy, and electrical stimulation. Behavioral and exercise therapy have limited efficacy, and pharmacotherapy has dose-limiting side effects. The Medtronic Interstim device targets the S3 sacral spinal nerve, but sacral stimulation is relatively non-selective due to the convergence of parasympathetic, sympathetic, and somatic innervation of lower urinary tract and other pelvic systems. Electrical stimulation of peripheral nerves for OAB may provide greater selectivity thereby increasing efficacy and reducing side effects. We hypothesized that electrical stimulation of pelvic nerve (PEL) can restore bladder capacity (BC) and voiding efficiency (VE) in the rat prostaglandin E2 (PGE2) OAB model. The aims of this study were: (1) quantify and compare functional (BC and VE) and neural (PEL and external urethral sphincter (EUS) activity) changes after intravesical installation of control (saline) and PGE2; and (2) measure the effects of pelvic stimulation on BC and VE post PGE2. Acute urethane (1.2 g/kg) anesthetized female Wistar rats (Charles Rivers Laboratories) were studied using *in vivo* cystometry (CMG). A PE-90 catheter was placed to measure bladder pressure and for intravesical infusion of saline and PGE2 (30, 60, and 100 μM). Bipolar electrodes were placed on EUS and PEL to record electromyogram (EMG) and electroneurogram (ENG) activity during CMGs. In a subset of trials, continuous PEL stimulation was delivered after PGE2 infusion at different amplitudes (0.8 and 2 times threshold to evoke PEL-EUS reflex) and frequencies (1 and 10 Hz). 100 μM intravesical PGE2 reduced both BC and VE compared to control. 100 μM intravesical PGE2 also reduced PEL ENG activity and increased EUS EMG activity. Low amplitude (0.8 times threshold) 10 Hz PEL stimulation restored both BC and VE to control levels. These findings suggest that PGE2 associated deficits in BC and VE may be due to a loss of coordinated control of both pelvic and pudendal nerves, and that PEL stimulation is a promising approach for treatment of OAB.

Disclosures: C.L. Langdale: None. **J.A. Hokanson:** None. **A. Sridhar:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **W.M. Grill:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.05/Z36

Topic: E.04. Autonomic Regulation

Support: NIH-NIDDK 2R01DK051369

NIH-NIDD 2R01DK060481

Title: Contributions of pituitary adenylate cyclase-activating polypeptide (PACAP)/receptor signaling to increased voiding frequency and somatic sensitivity in mice with urothelium-specific overexpression (OE) of nerve growth factor (NGF) in the urinary bladder

Authors: *B. M. GIRARD, S. MALLEY, M. E. MATHEWS, M. A. VIZZARD;
Anat. and Neurobio., Univ. Vermont col Med., Burlington, VT

Abstract: NGF-OE in the urothelium stimulates neuronal sprouting or proliferation in the urinary bladder, produces increased voiding frequency and non-voiding contractions, and results in increased referred somatic sensitivity. Additional NGF-mediated changes might contribute to the urinary bladder hyperreflexia and pelvic hypersensitivity observed in these transgenic mice such as upregulation of neuropeptide/receptor systems. NGF-OE in the urothelium was achieved through the use of a highly urothelium-specific, uroplakin II promoter. We examined PACAP, vasoactive intestinal polypeptide (VIP), and associated receptor (PAC1, VPAC1, VPAC2) transcripts or protein expression in urothelium and detrusor smooth muscle and lumbosacral dorsal root ganglia in NGF-OE and littermate wildtype (WT) mice using real-time quantitative reverse transcription-polymerase chain reaction and immunohistochemical approaches. Results demonstrate upregulation of PAC1 receptor transcript and PAC1-immunoreactivity (IR) in urothelium of NGF-OE mice whereas PACAP transcript and IR were decreased in urothelium. In contrast, VPAC1 receptor transcript was decreased in both urothelium and detrusor smooth muscle of NGF-OE mice. VIP transcript expression and IR was not altered in urinary bladder of NGF-OE mice. Given the presence of PAC1-IR fibers, the expression of PAC1 receptor expression in bladder tissues, and the abilities of PACAP to facilitate detrusor contractility, whether PACAP/receptor signaling contributes to bladder hyperreflexia and somatic sensitivity was evaluated. Intravesical administration of PACAP6-38 (300 nM) significantly ($p \leq 0.01$) increased bladder capacity (2.0-fold), intercontraction interval and void volume in NGF-OE mice. Intravesical instillation of PACAP6-38 also decreased filling pressure and peak micturition pressure in NGF-OE mice. PACAP6-38 had no effects on WT mice. Intravesical administration of PACAP6-38 (300 nM) significantly ($p \leq 0.01$) reduced pelvic sensitivity in NGF-OE mice but was without effect in WT mice. These studies demonstrate that additional NGF-mediated pleiotropic changes, such as modulation of neuropeptide/receptor systems, contribute to the increased voiding frequency and pelvic sensitivity observed in NGF-OE mice.

Disclosures: B.M. Girard: None. **S. Malley:** None. **M.E. Mathews:** None. **M.A. Vizzard:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.06/Z37

Topic: E.04. Autonomic Regulation

Support: NIH-NIDDK 2R01DK051369

NIH-NIDDK 2R01DK060481

Title: Pituitary adenylate cyclase-activating polypeptide (PACAP) expression in lower urinary tract pathways (LUT) with cyclophosphamide (CYP)-induced cystitis in PACAP promoter-dependent EGFP BAC transgenic mice

Authors: *M. A. VIZZARD[1], M. E. MATHEWS[1], S. MALLEY[1], B. M. GIRARD[1], K. M. BRAAS[1], J. A. WASCHEK[2], V. MAY[1];
[1]Neurolog. Sci., Univ. Vermont Col. Med., Burlington, VT; [2]Dept. of Psychiatry and Behavioral Sci., David Geffen Sch. of Med., Los Angeles, CA

Abstract: PACAP and associated receptors exist in rodent lower urinary tract (LUT); the majority derived from sensory neurons. PACAP expression in sensory neurons following nerve injury is changed; however, few studies have examined PACAP expression following inflammation. We have previously demonstrated an upregulation of PACAP expression in rodent micturition pathways following CYP-induced cystitis. We now examined the effects of CYP-induced cystitis (4 h, 48 h, chronic) in PACAP promoter-dependent EGFP BAC transgenic mice. We induced bladder inflammation in adult mice by injecting CYP intraperitoneally to produce acute (150 mg/kg; 4 h), intermediate (150 mg/kg; 48 h), and chronic (75 mg/kg; every third day for 10 days) cystitis. In control (no inflammation) animals, low basal expression of PACAP-EGFP+ fibers was present in the superficial DH at all segmental levels examined (L1, L2, L4-S1). Dorsal root ganglia (DRG; L1, L2, L6, S1) from control animals also exhibited PACAP-EGFP+ cells. After CYP-induced cystitis, PACAP-EGFP+ cells increased dramatically in spinal segments and DRG (L1, L2, L6, and S1) involved in micturition reflexes. Small diameter, PACAP-EGFP+ DRG cells co-localized with TRPV1- and TRPV4-IR. The density of PACAP-EGFP+ nerve fibers was increased in the superficial laminae (I-II) of the L1, L2, L6, and S1 DH. No changes in PACAP-EGFP+ nerve fibers were observed in the L4-L5 segments. PACAP-EGFP+ nerve fibers also increased in the lateral collateral pathway in L6-S1 spinal cord. Following CYP-induced cystitis, PACAP-EGFP+ urothelial cells were observed and the number of PACAP-EGFP+ urothelial cells increased with duration of cystitis. PACAP-EGFP+ urothelial cells were co-localized with TRPV4-IR. Changes in PACAP expression in LUT pathways after cystitis may play a role in altered visceral sensation (allodynia) and/or increased voiding frequency in the chronic inflammatory pain syndrome, interstitial cystitis/bladder pain syndrome.

Disclosures: **M.A. Vizzard:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH-NIDDK. **M.E. Mathews:** None. **S. Malley:** None. **B.M. Girard:** None. **K.M. Braas:** None. **J.A. Waschek:** None. **V. May:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.07/Z38

Topic: E.04. Autonomic Regulation

Support: VA RRD Award RX-000876-01-A1

Title: Cell therapy for neurogenic detrusor overactivity: proof of concept using intramural implantation of adrenal medullary chromaffin cells following chronic suprasacral spinal cord injury

Authors: ***M. O. FRASER**[1], J. M. BROOKS[2], D. J. DEGOSKI[2], P. C. DOLBER[3]; [1]Dept. of Surgery, Duke Univ. and Durham VA Med. Centers, Durham, NC; [2]Inst. for Med. Res., Durham, NC; [3]Univ. of Texas Med. Br., Galveston, TX

Abstract: Objectives: Suprasacral spinal cord injury (SCI) is often occasioned by the development of high pressure non-voiding contractions (NVC). NVC may result in significant morbidities, such as ureteral reflux, incontinence and autonomic dysreflexia. Previous work from our laboratory has demonstrated that β3-adrenergic receptor agonists are effective in reducing NVC number and amplitude in chronic SCI rats. To test whether stimulation of β-adrenergic receptors (BAR) by endogenous catecholamines as a result of cell therapy would similarly inhibit NVC, we transplanted adrenal medullary chromaffin cells (AMCC) into the bladder walls of SCI rats and examined their survival and function. Methods: Recipient female Lewis rats underwent SCI at T9-10 and their bladders were manually expressed 2X daily for 4-6 weeks prior to AMCC or Media bladder wall injections. On the day of bladder wall injections, adrenal medullas were harvested and AMCC isolated from donors. AMCC were injected in 4-6 bladder wall sites for total cell loads of 10.9 ±1.6 x 10^5 cells/bladder (n=10). Media animals (C) received 50 µl of culture medium (n=9). The animals recovered for 1-2 weeks prior to cystometric evaluation. On the day of cystometry, rats received jugular and transvesical catheters. Following 1 hour recovery, saline infusion (0.1 ml/min) into the bladder was initiated. One hour later, the animals received injections of vehicle and, 30 min later, a cocktail of BAR

antagonists (0.5 mg/ml propranolol + 1 mg/ml SR-59230A). Perfusion fixed bladders were harvested and processed for tyrosine hydroxylase (TH) immunofluorescence. Results: Treatment with BAR antagonists resulted in a ~2.5 fold increase in the number of NVC events in AMCC rats ($P<0.0001$; 2-Way ANOVA), while no effect was seen in C. Mean values for the AMCC rats were ~40% lower and higher during the vehicle control and the BAR blockade periods than C, respectively. Finally, numerous TH+ AMCC cells were detected in bladders from AMCC rats. Conclusions: Cell therapy using catecholamine (norepinephrine and epinephrine) producing cells holds promise for the treatment of NVC due to suprasacral SCI. AMCC may be harvested from the patients' own adrenal medullae, or autologous stem/progenitor cells may be converted into catecholamine producing cells for this purpose.

Disclosures: M.O. Fraser: None. **J.M. Brooks:** None. **D.J. Degoski:** None. **P.C. Dolber:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.08/Z39

Topic: E.04. Autonomic Regulation

Support: Fondecyt #1140776

Title: Early-life dysbiosis and its effects on anxiety-like behaviors in the infant rat

Authors: *J. A. BRAVO[1], E. PONCE-GUEQUEN[2], C. BARRERA-BUGEÑO[2], J. ESCOBAR-LUNA[2], M. GOTTELAND[3], M. JULIO-PIEPER[2];
[1]Pontificia Univ. Catolica de Valparaiso, VALPARAISO, Chile; [2]Inst. de Química, Facultad de Ciencias, Pontificia Univ. Católica de Valparaíso, Valparaíso, Chile; [3]Dept. de Nutrición, Facultad de Medicina, Univ. de Chile, Santiago, Chile

Abstract: Intestinal colonization of the neonatal gut begins at the birth canal and continues as the newborn comes in contact with the mother (e.g.: breastfeeding). Therefore, interventions in the maternal gut microbiota, like the use of antibiotics in the perinatal period can modify the infant's microbiota as well. On the other hand, intestinal dysbiosis has been shown to affect human and animal behavior, thus establishing what is now recognized as the microbiota-gut-brain axis. Evidence shows that acquisition of a balanced gut microbiota in early-life is important for the adequate development of behavioral and physiological responses to stress. For instance, germ-free mice have reduced anxiety-like behaviors despite producing an exaggerated release of corticosterone upon a stressful stimuli. In addition, non-absorbable wide-spectrum antibiotics reduce anxiety-like behaviors in adult rodents. Notably, all these observations have been made in adult animals, and very little is known about the effects of gut dysbiosis in the behaviour at

young age. Thus, in order to test this we administered a mixture of non-absorbable wide spectrum antibiotics (neomycin 5 mg/ml, bacitracin 5 mg/ml, pimaricin 1.25microg/ml and vancomycin 0.5 mg/ml) in the drinking water of pregnant Sprague-Dawley dams, starting at three days before parturition and maintained until post-natal day (PND) 7, while control dams were given water alone. On PND 21 pups were weaned and behavioral testing begun on PND 22. Open field test revealed that male, but not female pups whose mother was exposed to antibiotics spent more time in the central area of the apparatus in comparison to control rats. On day 23, elevated plus maze test revealed that both female and male pups from dams exposed to antibiotics spent more time in the open arms (OA) and had an increased number of entries into the OA too, in comparison to control rats. On PND24 rats were subjected to the forced swim test, however there were no differences between treatments. These findings suggest that alterations in the bacteria colonizing the gastrointestinal tract during early-life (PND1-7) affect anxiety-like behaviors in infant rodents, but do not induce depression-like behaviors. These findings strongly support the notion that acquisition of a balanced microbiota is important for the development of adequate stress-related behaviors. Moreover, these changes are observable during infancy suggesting a potential timeframe when interventions can be made in order to prevent the occurrence of psychiatric-related disorders later in life.

Disclosures: J.A. Bravo: None. **E. Ponce-Guequen:** None. **C. Barrera-Bugeño:** None. **J. Escobar-Luna:** None. **M. Gotteland:** None. **M. Julio-Pieper:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.09/Z40

Topic: E.04. Autonomic Regulation

Support: Fondecyt #1130213

Conicyt #79112017

Title: *Ex vivo* effects of fluoxetine on the rat intestinal barrier: relevance to disorders of the brain-gut axis

Authors: *M. JULIO-PIEPER, C. GONZALEZ-ARANCIBIA, M. P. GONZALEZ-TORO, J. A. BRAVO;
Instituto De Quimica, Pontificia Universidad Catolica De Valparaiso, Valparaiso, Chile

Abstract: Psychological stress is frequently associated with gastrointestinal (GI) symptoms including dysmotility and increased pain perception. The term “brain-gut axis disorder” has been used to describe a number of pathological situations where comorbidity of both systems has been well established. One classic example is irritable bowel syndrome, highly comorbid with

depression and anxiety. However, it is still unknown whether one condition leads to the other, or if they are both unrelated consequences of a primary event. Interestingly, one GI alteration that may be induced by acute or chronic psychological stress is increased intestinal permeability. In a healthy organism, the gut barrier prevents translocation of noxious luminal substances and microorganisms. Incorporation of luminal contents to the endothelial compartment may lead to local or systemic inflammatory disorders and possibly alterations in enteric nerve function, potentially worsening the patient's condition. During the last few years, antidepressant treatment has been used to provide relief to patients suffering from brain-gut axis disorders, with the main focus in the GI side of these conditions being restoration of normal motility and reduction in abdominal pain. However, little is known about the potential actions of antidepressant drugs on intestinal barrier function. Selective serotonin reuptake inhibitors (SSRIs) have anti-inflammatory effects and can inhibit toll-like receptor activity. Because inflammatory and immune mediators are important modulators of epithelial permeability, we aimed to investigate whether the SSRI fluoxetine is able to improve intestinal barrier function by acting directly on the intestinal tissue. We applied fluoxetine (0, 0.5, 5 or 50 microg/mL) on rat ileal and colonic preparations and examined tissue permeability *ex vivo*. The drug did not affect the permeation of macromolecules (4.4 and 40 kD dextrans) on either tissue along the three-hour experiment. However, the highest dose of fluoxetine significantly reduced transepithelial electrical resistance of both ileum and colon by 1 hour of treatment, indicating an increase of tissue permeability to electrolytes and/or small molecules. Our results show that a short-term *ex vivo* fluoxetine treatment does not strengthen intestinal barrier function; moreover the highest dose (50 microg/mL) induced barrier disruption. Although patients with major depression treated with fluoxetine have lower plasma levels than the doses tested here, the effects of long-term *in vivo* fluoxetine administration on intestinal permeability remain to be investigated.

Disclosures: M. Julio-Pieper: None. **C. Gonzalez-Arancibia:** None. **M.P. Gonzalez-Toro:** None. **J.A. Bravo:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.10/Z41

Topic: E.04. Autonomic Regulation

Support: Craig H. Neilsen Foundation (PI: SA Sisto #284755)

Thomas Hartman Center for Parkinson's Research at Stony Brook University - Pilot Award (W. Collins)

Title: Serial Cystometry Measurements- a novel method for evaluation of lower urinary tract function in adult female sprague-dawley rats

Authors: F. QURESHI[1], P. KUNG[2], *S. A. SISTO[3], W. F. COLLINS, III[2];
[1]Hlth. and Rehabil. Science, Sch. of Hlth. Technol. and Mgmt., [2]Neurobio. and Behavior, [3]PhD in Hlth. & Rehab Sci., Stony Brook Univ., Stony Brook, NY

Abstract: Lower urinary tract (LUT) dysfunction is a common sequelae to many neurological disorders including spinal cord injury (SCI). The aim of the present study was to develop a method for reliable serial measurements of LUT function, including simultaneous bladder pressure and external urethral sphincter (EUS) activity over 12-16 weeks. Cystometry, combined with EUS EMG recording, was performed weekly on adult female Sprague-Dawley rats (225-250 g, n=8) under Ketamine/Xylazine anesthesia (90/10 mg/kg, i.p.). For each session, a sterile catheter (PE50) was inserted transurethrally into the bladder and connected in series to a pressure transducer to record bladder pressure and to a 60cc syringe attached to a syringe pump. EUS EMG recordings were made by inserting two sterile fine wire electrodes (A-M Systems; 50 μm insulated stainless steel) percutaneously into or near the EUS muscle using a 30 gauge hypodermic needle. Repetitive micturition events were elicited by continuous infusion of saline into the bladder (5-8 ml/hr, room temperature) and recordings of bladder pressure and EUS EMG activity were acquired. Each rat underwent simultaneous measurements of bladder and EUS once a week for three consecutive weeks where threshold bladder pressure (TBP), peak bladder pressure during contraction (PBP), duration of bladder contraction, average EUS peak amplitude, sustained EUS contraction after bursting, area under EUS EMG normalized by average peak (NAEUS), burst duration, and number of bursts per EUS contraction were captured. Reliability testing using intra-class correlation coefficient (ICC) test showed significant PBP (ICC=0.46, p=0.001), TBP (ICC=0.423, p=0.0001) and NAEUS (ICC=0.37,p=0.001) across days and rats. Our results show that this novel method of repeated measurements of LUT over several weeks is reliable producing consistent data for PBP, TBP and NAEUS, especially if detrusor sphincter dysynergia (DSD) is expected. We propose that this method can be used to follow changes in recovery of LUT function after chronic SCI, particularly for quantitative evaluation of DSD.

Disclosures: F. Qureshi: None. **P. Kung:** None. **S.A. Sisto:** None. **W.F. Collins, III:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.11/Z42

Topic: E.04. Autonomic Regulation

Support: F32 DK098904

R01 NS050514

Title: Urethral sensory neuron activation by flow: electrophysiological quantification and modeling

Authors: *Z. C. DANZIGER, W. M. GRILL;
Biomed. Engin., Duke Univ., Durham, NC

Abstract: It is necessary to understand the neural encoding of sensation in the urethra to diagnose better, clarify etiology, and develop treatments for neuropathy-induced voiding dysfunction. We quantified the response of the sensory branch of the pudendal nerve (which densely innervates the urethra) to urethral pressure and flow. Physiological saline was passed through the urethra of urethane anesthetized Sprague-Dawley rats (without filling the bladder) via a suprapubic catheter to control directly flow through the urethra, which allowed us to expose the urethra to a wide range of flow rates and pressures to characterize the sensory response. We found that the primary stimulus driving urethral afferents was large, positive changes in intraurethral pressure. Constant pressures produced elevated levels of sensory activation, but these responses were of much lower magnitude and of greater variability than responses to changes in the pressure, indicating that pressure derivatives are the critical stimulus for these afferents. Notably, only increases (not decreases) in urethral pressure caused these large afferent responses. We also systematically mapped the time courses of the sensory responses and show, for the first time, that there is a significant long term (minutes) neural accommodation to changes in urethral pressure. The reduction of the pudendal sensory response caused by accommodation is of similar magnitude to the effect of changing the applied flow rate, indicating that this accommodation is of a physiologically relevant magnitude. We hypothesize that the phasic contraction of the external urethral sphincter observed during voiding in some rats may be linked to the fact that urethral afferents are selectively responsive to pressure derivatives because phasic bursting generates large and frequent pressure changes that amplify pudendal sensory activation. We present evidence for this from experiments where the pudendal motor branch, contralateral to the pudendal recording site, was stimulated to elicit controlled urethral sphincter contractions during applied flow, which resulted in pressure oscillations and increases sensory response. Further, experiments using the paralytics phentolamine and propranolol confirm that the neural responses and their accommodation are neurally mediated, rather than the result of an active reflex modulating the intraurethral pressure. Finally, we developed a compact mathematical model that predictively maps measured pressure into pudendal afferent response. This description of sensory signaling in the urethra provides a foundation to understand disruptions that may occur following trauma or disease.

Disclosures: Z.C. Danziger: None. **W.M. Grill:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.12/Z43

Topic: E.04. Autonomic Regulation

Support: Craig H. Neilsen Foundation Grant 314980

Title: Chronic monitoring of lower urinary tract neurophysiology via sacral dorsal root ganglia

Authors: A. KHURRAM, S. E. ROSS, A. A. A. JIMAN, Z. J. SPERRY, C. M. MAHAR, *T. M. BRUNS;
Univ. of Michigan, Ann Arbor, MI

Abstract: Understanding lower urinary tract (LUT) neurophysiology is important for developing a closed-loop bladder neuroprosthesis in which information about the bladder state can be used to control and improve stimulation. However, literature on long-term recordings of afferent neurophysiology of the LUT is lacking. In contrast to previous studies in which spinal electrodes or nerve cuffs have been individually implanted to monitor and stimulate the LUT in short-term studies, we are developing an integrated approach of chronically recording afferent activity of the LUT at dorsal root ganglia (DRG) in a feline model. Two 32-channel microelectrode arrays were implanted in sacral DRG after a laminectomy for recording afferent activity while a nerve cuff was implanted on the pudendal nerve for stimulation. A dual-lumen supra-pubic bladder catheter was implanted to provide access for controlling bladder volume and monitoring pressure. The array connectors and cuff wires were contained in an external housing that was mounted percutaneously to the iliac crests. Periodic data collection sessions were held after implant surgery while the cat was sedated with dexmedetomidine. Bladder filling and emptying trials were done where the bladder was infused with saline or emptied through the bladder catheter. We have identified and tracked unique bladder sensory neurons across sessions (average firing rate of 2.6 spikes/s, range 1-12 spikes/s; bladder pressure correlation coefficients of 0.6 and higher). We also performed stimulation-driven bladder voiding trials in which the pudendal cuff was stimulated at 5-30 Hz. During these trials, effective bladder emptying (75-85% efficiency; up to 16 mL in 20 s) has occurred in which bladder and urethra flow afferents were identifiable. The urethra flow afferents fired at 3 spikes/s during initial leakage and up to 48 spikes/s during maximal urine flow. Our setup allows us to track LUT neurons over time and examine their characteristics. From these data, we hope to gain a better understanding of the LUT afferent neurophysiology, which can be used as a control signal for driving stimulation in a closed-loop bladder neuroprosthesis.

Disclosures: A. Khurram: None. **S.E. Ross:** None. **A.A.A. Jiman:** None. **Z.J. Sperry:** None. **C.M. Mahar:** None. **T.M. Bruns:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.13/Z44

Topic: E.04. Autonomic Regulation

Title: Assessing gastrointestinal actions of commonly prescribed pharmacologic compounds using the gastrointestinal motility monitor (gimm) *in vitro* assay

Authors: *M. KLINGER, M. M. MCGILL, N. M. WIGHTON, S. R. BRUNO, G. M. HERRERA;
Catamount Res. and Development, Inc., Saint Albans, VT

Abstract: Functional gastrointestinal disorders such as irritable bowel syndrome, colitis, and chronic constipation affect millions of people. In addition to these disorders, many patients report adverse gastrointestinal affects from the use of commonly prescribed medications, including anti-hypertensives, opiate-based pain medications, and anti-depressants. These side effects cause many patients to discontinue treatment. Constipation is a persistent problem in human health, and still requires further investigation. Using the Gastrointestinal Motility Monitor (GIMM) *in vitro* pellet propulsion assay, we measured the effects of four compounds known to affect colonic function and motility in the human. These are the calcium channel blocker verapamil (0.1 - 10 uM), the opioid receptor agonist morphine (0.01 - 1 uM), the selective serotonin reuptake inhibitor fluoxetine (10-40 uM), and the norepinephrine/dopamine reuptake inhibitor bupropion (20-100 uM). Distal colons were isolated from guinea pigs following euthanasia, and were immersed in physiological saline solution (PSS, 37 °C). In each segment of colon, three baseline motility trials were completed by placing a fecal pellet inside the oral end, followed by three trials in the presence of test compounds. Through natural peristaltic motion, the pellet moved through the colon and its velocity was recorded by the GIMM. Colon segments with an average baseline motility below 1 mm/s were excluded from the study. Verapamil (1 μM) significantly increased pellet velocity by 50% as compared with baseline, while verapamil at 10 μM completely abolished motility. Morphine (1 μM) significantly reduced pellet velocity by 30%, and morphine at a concentration of 10 μM reduced motility by 90%. Fluoxetine (20 and 30 μM) reduced pellet propulsion velocity by 50%, and velocity was reduced by 60% with fluoxetine at 40 μM. Bupropion (100 μM) reduced pellet velocity by 90%. In conclusion, the velocity of fecal pellet propulsion in guinea pig distal colon is a useful *in vitro* assay to assess actions of pharmacological compounds on the gut, and this may be useful for drug development.

Disclosures: **M. Klinger:** A. Employment/Salary (full or part-time):; Catamount Research and Development. **M.M. McGill:** A. Employment/Salary (full or part-time):; Catamount Research and Development. **N.M. Wighton:** A. Employment/Salary (full or part-time):; Catamount Research and Development. **S.R. Bruno:** A. Employment/Salary (full or part-time):; Catamount Research and Development. **G.M. Herrera:** A. Employment/Salary (full or part-time):; Catamount Research and Development.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.14/AA1

Topic: E.04. Autonomic Regulation

Support: Medtronic Research Grant

Title: A functional analysis of the influence of pelvic nerve on the micturition reflex in rats with acetic acid induced cystitis

Authors: *X. SU, J. E. AGRAN, D. E. NELSON;
Medtronic, Minneapolis, MN

Abstract: Bladder hypersensitivity is associated with the over excitation of the pelvic nerve (PN) including both the afferent-ascending pathway, which triggers urgency and the parasympathetic efferent-descending pathway, which induces the detrusor contraction. In this study we did a functional analysis of cystometric parameters with or without PN injury in rats with acetic acid (a.a.) induced cystitis. Male rats were anesthetized (urethane, IP 1.2 g/Kg) and cannulated with a bladder catheter via the bladder dome for 0.3% a.a. infusion (3 ml/hr). The cystometric parameters were compared in 3 groups of cystitis rats that received different levels of PN manipulation, 1) rats that received no PN manipulations (n=7), 2) rats that received partial PN injury using 4-6 gentle longitudinal stretches of the nerve bilaterally with a cotton tipped applicator (n=7), and 3) rats that received complete successive PN transection (unilaterally, followed by bilaterally) using preplaced silk threads (n=11). Intravesical a.a. infusion induced a hypersensitive bladder; the inter-contraction interval (ICI) was 197 ± 41 s (mean ± SEM). Partial PN injury significantly increased ICI to 404 ± 90 s (P=0.03, unpaired t-test), which was observed for up to 3 hrs. The increased ICI (or infused volume) was accompanied by an increase in both void volume and void duration. The maximal pressure was unchanged indicating the void functions were not impacted by partial PN injury. Unilateral PN transection produced total incontinence in 1/11 rat and partial incontinence in 3/11 rats. In addition, unilateral PN transection produced an immediate inhibition on bladder afferent function, increasing the ICI, from 497 ± 59 s to 892 ± 93 s (p=0.002, paired t-test) and threshold pressure, from 28 ± 2 mmHg to 30 ± 2 mmHg (p=0.008, paired t-test). However the efferent voiding function was decreased as reflected by mismatch of the void volume (0.43 ± 0.1 ml) and infused volume (0.74 ± 0.1 ml, p=0.03, paired t-test). Bilateral PN transection abolished voiding in all rats. In the rat cystitis model, the bladder hyperactivity was used to mimic the human condition of detrusor overactivity. This excitatory effect was significantly attenuated by partial PN injury when both PNs were intact. Intact PNs are critical for maintenance of normal voiding functions and an unilateral injury of the PN may significantly affect the micturition function. These results emphasize the important role that PNs play in normal bladder functions and their potential involvement in bladder dysfunction such as overactivity.

Disclosures: **X. Su:** A. Employment/Salary (full or part-time):; Medtronic, plc. **J.E. Agran:** A. Employment/Salary (full or part-time):; Medtronic, plc. **D.E. Nelson:** A. Employment/Salary (full or part-time):; Medtronic, plc.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.15/AA2

Topic: E.04. Autonomic Regulation

Support: CONACyt fellow grant 290817

CONACyT project 178027

Title: "Evaluation of the mechanism involved in the gastro protective effect of DHA (docosahexaenoic acid) in the indomethacin-induced gastric injury model in mice"

Authors: *E. A. PINEDA[1], A. E. CHÁVEZ-PIÑA[2];
[1]Lab. de Farmacología de la Sección de estudios de posgrado e investigacio, Escuela Nacional De Medicina Y Homeopatía Del Inst., México, Mexico; [2]ENMyH, Natl. Polytechnic Inst. (IPN), México, Mexico

Abstract: Introduction. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are widely used as analgesic and anti- inflammatory drugs for treatment of a variety of diseases characterized by inflammatory conditions; however, it has been reported that the use of NSAIDs causes severe adverse effects including erosions, bleeding and perforation with blood and protein loss in the gastrointestinal tract (GI), these side-effects limit their use. Besides the prostaglandin inhibition, gastric tissue damage by indomethacin is generated by others prostaglandin-independent mechanisms including induction of proinflammatory TNF-α expression, ICAM-1 expression, neutrophil infiltration and microvascular injury. Previous studies reported the gastroprotective effect of DHA (docosahexaenoic acid), but the mechanism and molecules involves in this effect have not been fully explained. The aim of this study was to examine the molecules and mechanism implicated in the gastroprotective effect of DHA. **Methods.** Balb/c mice received oral administration of DHA (3, 10, 30 and 100 mg/kg), and 2 h later, gastric damage was induced by a single oral dose of indomethacin (30 mg/kg). Five hours later, mice were anesthetized and euthanized by cardiac puncture. The stomach was removed and fully extended, then a macroscopic analysis of the total gastric lesion area (mm2) for each mouse was performed; a sample of gastric mucosa was taken and the leukocyte infiltration (by measuring Myeloperoxidase, Leukotriene B4 and histology study) and TNF-α levels were assessed. **Results and Discussion.** Our results showed that DHA treatment induces a gastroprotective effect in a dose-dependent manner against indomethacin-induced gastric injury. DHA pre-treatment

exhibits the ability to reduce the leukocyte infiltration in gastric tissue through the modulation of myeloperoxidase (MPO), LTB4 and the decreasing of TNF-α molecule. Our results suggest that DHA modulates the molecules involved in neutrophil infiltration induced by indomethacin administration and some pro-inflammatory molecules. In conclusion, DHA pre-treatment showed a gastroprotective effect against indomethacin-induced gastric injury effect through the modulation of leukocyte infiltration and others molecules implicated in inflammation process, therefore it may be a therapeutic resource to limit NSAIDS side-effects in gastrointestinal tract.
Acknowledgments. the authors acknowled the support provide by the National Council Science and Technology (Project CONACyT 178027). and the National Polytechnic Institute (Project SIP-20150031).

Disclosures: E.A. Pineda: None. **A.E. Chávez-Piña:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.16/AA3

Topic: E.04. Autonomic Regulation

Support: Basic Science Research Programs through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013011219)

Basic Science Research Programs through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2013056735)

Title: Anti-inflammatory effects of acupuncture stimulation via the vagus nerve

Authors: *H. LIM[1], I. CHANG[2], J. LEE[2], K. KIM[2], C.-Y. LEE[3], U. NAMGUNG[2];
[2]Oriental Med., [3]Microbiology and Biotech., [1]Daejeon Univ., Daejeon, Korea, Republic of

Abstract: Although acupuncture therapy is widely used in traditional Asian medicine for the treatment of diverse internal organ disorders, its underlying biological mechanisms are largely unknown. Here, we investigated the functional involvement of acupuncture stimulation (AS) in the regulation of inflammatory responses. TNF-α production in mouse serum, which was induced by lipopolysaccharide (LPS) administration, was decreased by AS at the zusanli acupoint (stomach36, ST36). In the spleen, TNF-α mRNA and protein levels were also downregulated by AS and were recovered by using a splenic neurectomy. c-Fos, which was induced in neurons in the nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus nerve (DMV) by LPS and acupuncture given manually and electrically, was further increased by focal administration of the AMPA receptor blocker CNQX and the purinergic receptor antagonist PPADS in the area of the dorsal vagal complex (DVC). TNF-α levels in the spleen were decreased by CNQX and PPADS treatments, implying the involvement of inhibitory neuronal

activity in the DVC. AS in unanesthetized animals generated similar patterns of c-Fos induction in the DVC neurons and of splenic TNF-α production. Thus, our data suggest that the therapeutic effects of acupuncture may be mediated through vagal modulation of inflammatory responses in internal organs.

Disclosures: **H. Lim:** None. **I. Chang:** None. **J. Lee:** None. **K. Kim:** None. **C. Lee:** None. **U. Namgung:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.17/AA4

Topic: E.04. Autonomic Regulation

Support: Craig H. Neilsen Foundation Grant 314980

Title: Quantification of hysteresis in bladder afferent neurons in response to changes in bladder pressure

Authors: ***S. E. ROSS**[1], Z. J. SPERRY[1], C. M. MAHAR[2], T. M. BRUNS[1];
[1]Biomed. Engin., [2]Nuclear Engin. and Radiological Sci., Univ. of Michigan, Ann Arbor, MI

Abstract: For patients with bladder dysfunction refractory to standard treatments, electrical stimulation of lower urinary tract nerves may be an alternative approach for controlling bladder function. A closed-loop system that provides feedback on the bladder state and delivers stimulation when needed may be advantageous over currently available devices. We are evaluating an interface with sacral dorsal root ganglia (DRG), at which we can monitor bladder afferent signals from the pelvic nerve and stimulate pudendal nerve pathways that drive bladder control spinal circuits. A key challenge is developing a robust model to estimate bladder pressure from the firing rate activity of bladder afferent neurons. Though linear regression can give estimates of the bladder pressure from bladder afferent firing rates, these linear models often yield lower quality fits during subsequent time periods. A major factor in this model inadequacy is that the firing rate of a bladder afferent neuron is typically different during the rising and falling phases of a bladder contraction. Previous studies have noted this firing rate-pressure hysteresis, but it has not been quantified (as far as we know). Our goal here is to examine bladder afferent neuron hysteresis, towards developing a model that takes into account this dynamic relationship. Bladder afferent activity was recorded with microelectrode arrays inserted into the first and second sacral DRG of intact male cats. In four acute experiments, 59 bladder afferents were identified based on their characteristic responses to pressure (correlation coefficient > 0.20) during infusion under alpha-chloralose anesthesia. We calculated maximum (Hmax) and mean (Hmean) hysteresis values for each afferent as the maximum and mean ratio between the firing

rate difference at each pressure (in 5 cmH2O bins) and the overall firing rate range. The hysteresis value range of 0 - 1 represents zero to maximal hysteresis. Across afferents we observed a mean Hmax of 0.78 ± 0.24 (mean ± standard deviation), with 28 neurons greater than 0.80, and a Hmean of 0.37 ± 0.31. These results indicate that correlated bladder afferents show moderate to high hysteresis. Advanced state models, such as neural networks, that can incorporate this non-linear relationship may lead to consistent estimates of bladder pressure.

Disclosures: **S.E. Ross:** None. **Z.J. Sperry:** None. **C.M. Mahar:** None. **T.M. Bruns:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.18/AA5

Topic: E.04. Autonomic Regulation

Support: 1R21NS086413

Title: Developmental analysis of MET-EGFP transgene cell phenotypes and projection targets in the brainstem Vagal Motor Complex

Authors: *A. K. KAMITAKAHARA, H.-H. WU, P. LEVITT;
Children's Hosp. Los Angeles, Los Angeles, CA

Abstract: Recent meta-analysis data suggests that children with Autism Spectrum Disorder (ASD) are more than four times as likely to have comorbid gastrointestinal dysfunction (GID). One genetic point of convergence for both ASD and GID is a common promoter variant in the human *MET* receptor tyrosine kinase (*MET*) gene, which reduces MET expression, and is highly enriched in families of children with both ASD and co-occurring gastrointestinal symptoms. MET signaling has been demonstrated to be critically important for mouse neocortical and hippocampal dendritic elaboration and synaptogenesis; however it is unknown whether MET is also required for the development of autonomic brainstem circuits that modulate gastrointestinal function. To this end, we are characterizing the expression of MET in developing vagal visceromotor neurons in the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus (nA) in Met^{EGFP} mice using immunohistochemistry for both EGFP and MET protein. MET is highly expressed by a subset of neurons in the lateral DMV and the vast majority of nA neurons beginning around E11.5, shortly after these neurons are generated. Vagal MET^{EGFP} expression is sustained into the first two postnatal weeks, after which it is expressed only at moderate levels. All MET^{EGFP} neurons of the DMV and nA are cholinergic by analysis of choline-acetyltransferase (ChAT) co-expression. In addition, MET^{EGFP} neurons in the DMV are immunoreactive for the neuropeptide, cocaine- and amphetamine related transcript (CART). *In situ* hybridization further revealed that the MET ligand, hepatocyte growth factor (HGF), is

expressed prenatally in both the esophagus and intestinal submucosa, suggesting that target-derived HGF may impact the innervation of these regions of the gastrointestinal tract by MET-expressing neurons from the brainstem. Experiments are currently underway using whole mount immunostaining in conjunction with tissue clearing to determine the innervation patterns of MET-expressing neuronal projections to the gastrointestinal tract. Additionally, RNA sequencing of sorted vagal MET^{EGFP} expressing neurons will identify novel gene targets involved in the development of the circuits controlling gastrointestinal function.

Disclosures: **A.K. Kamitakahara:** None. **H. Wu:** None. **P. Levitt:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.19/AA6

Topic: E.04. Autonomic Regulation

Support: Seed grant ICORD

Title: "Fast and slow": bowel dysfunction in spinal cord injured animals

Authors: *B. FRIAS[1], S. GOLBIDI[2], I. LAHER[2], A. KRASSIOUKOV[1];
[1]ICORD, Vancouver, BC, Canada; [2]Dept. of Anaesthesiology, Pharmacol. and Therapeut., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Introduction: Bowel dysfunction is among a broad range of autonomic abnormalities following spinal cord injury (SCI). SCI-patients experience symptoms, such as constipation, fecal incontinence and pain, which have a profound impact on their quality of life. These symptoms vary based on the type and level of SCI. Autonomic dysreflexia (AD), a life-threatning condition characterized by sudden increases in blood pressure and bradycardia, often occurs in patients with injuries above T6 level. AD can be triggered by non-noxious and noxious stimuli, such colon or bladder distention. Therefore, this study aimed to unravel the contribution of cardiovascular system impairment for bowel dysfunction. For that, an animal model of colorectal distention (CRD) will be used for induction of clinically relevant AD-symptoms. Methods: Male Wistar rats were assigned to groups (n=6/group): Sham, T3-SCI, T3-SCI+CRD, T10-SCI and T10-SCI+CRD. The assessment of induced-AD was performed by implanting a telemetry device in the carotid artery followed by CRD. For evaluation of colonic motility, the total number of fecal pellets and total gastrointestinal transit time (TGTT) were assessed at baseline and weekly after SCI for 4weeks. Animals were then sacrificed and 1cm of the proximal and distal colon was collected for wire-myography. Results: Chronic T10-SCI+CRD animals did not develop AD, in contrast to T3-SCI+CRD. The number of fecal pellets was decreased post-SCI ($p<0.05$ vs. Sham) but remained unchaged with CRD. TGTT was increased in following SCI

($p<0.05$ vs. Sham) and CRD caused early increase of TGTT at 1week ($p<0.05$ vs. T3-SCI/T10-SCI).At 4wks post-SCI, decreased contractility was registered in distal colon of T3-SCI ($p<0.0001$ vs. Sham/T10-SCI) and T10-SCI animals ($p<0.0107$ vs. Sham). CRD caused different effects in contractility depending on level of injury. In the proximal colon, contraction was decreased after SCI. T10-SCI+CRD animals presented decreased contraction. Conclusion: The data demonstrates that the level of SCI plus autonomic stress of CRD can contribute to the severity and early onset of bowel dysfunction.

Disclosures: **B. Frias:** None. **S. Golbidi:** None. **I. Laher:** None. **A. Krassioukov:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.20/AA7

Topic: E.04. Autonomic Regulation

Support: Craig Neilsen Foundation

Missouri Spinal Cord Injuries Research Program

Title: Characterization of cholinergic neurotransmission of bladder-innervating postganglionic neurons in mouse major pelvic ganglia through molecular, pharmacological and physiological measures

Authors: *C. KYI[1], D. J. SCHULZ[2];
[1]Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO; [2]Biol. Sci., Univ. of Missouri, Columbia, MO

Abstract: Major pelvic ganglia (MPG) of mouse contain postganglionic neurons innervating pelvic organs such as urinary bladder, colon and the genitals. Pelvic ganglia receive cholinergic parasympathetic inputs from the preganglionic neurons in the sacral cord through pelvic nerve, and sympathetic cholinergic inputs from those in the lumbosacral cord through hypogastric nerve via inferior mesenteric ganglion. The storage function of micturition is mostly under tonic inhibitory control of sympathetic nervous system and voiding function is mostly under the control of parasympathetic nervous system. The axons from the pelvic ganglion synapse onto postganglionic neurons on the bladder detrusor, releasing acetylcholine from parasympathetic axons and noradrenaline from sympathetic axons. Data from our previous study showed pairwise correlated decrease in mRNA expressions among α3&β2 as well as α3 &β4 cholinergic receptor subunits throughout postnatal development suggesting changes cholinergic transmission in the postganglionic MPG neurons. We characterized the cholinergic ganglionic transmission at bladder-innervating MPG neurons in normal adult mice. We retrograde-labelled populations of bladder-innervating neurons in the MPG. Then, we performed absolute quantitation of multiple

receptor transcript copy number for each sample, and employed multiple correlation analyses to detect patterns in mRNA levels across all genes of interest. With these data, we sought to determine whether there are underlying relationships among cholinergic receptor mRNA levels in the bladder-innervating neurons at the MPG. We also performed pelvic nerve stimulations and recorded EPSPs from the neuronal cell bodies using intracellular sharp electrodes and characterized the Ach-activated currents using agonists and subunit-specific receptor antagonists. These data will subsequently provide a baseline of comparison to examine changes cholinergic transmission in injury models such as spinal cord injury.

Disclosures: C. Kyi: None. **D.J. Schulz:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.21/AA8

Topic: E.04. Autonomic Regulation

Support: NIH-ENDURE

NSF-IOS

University of Puerto Rico

Title: Characterization of neuroendocrine cell subpopulations in the digestive tract of the echinoderm Holothuria glaberrima

Authors: *M. A. LEFEBRE[1], J. E. GARCIA-ARRARAS[2];
[1]Inter American Univ. of Puerto Rico - Bayamon, Corozal, PR; [2]Dept. of Natural Sci., Univ. of Puerto Rico - Rio Piedras Campus, Rio Piedras, Puerto Rico

Abstract: Echinodermata is a phylum of marine animals that lie at the basal branch of the deuterostome evolutionary branch. Their nervous system has a radial symmetry that includes five radial nerve cords that join anteriorly in a nerve ring. Recent work by our laboratory and others has explored the different echinoderm nervous components. Here we evidence the neurosecretory component of their digestive system. Immunohistological techniques, were used to identify the neuroendocrine cells of Holothuria glaberrima in four digestive tract regions: the esophagus, descending small intestine, ascending small intestine, and the large intestine. Antibodies that recognize the STARD10 protein, Calbindin, the neuropeptide GFSKLYFamide, GABA and the transcription factor Nurr1 were used to observe, describe and quantify the neuroendocrine cells. Our results show that there are at least two different subpopulations of neuroendocrine type cells associated with the luminal tissue and one more neuron-like subpopulation in the connective tissue of the gastrointestinal tract of H. glaberrima. The

neuroendocrine cells found in the luminal epithelium, were labeled with anti-Calbindin and anti-GFSKLYFamide. The calbindin expressing population accounted for about 4% of the luminal cells while those expressing the neuropeptide accounted for 25 of the luminal cells. These cells also differed in other properties. The cells labeled with anti-GFSKLYFamide extended fibers into the connective tissue, while those labeled with anti-calbindin had limited accounted for about 2.5% of the luminal cells and had projections that were contained within the luminal epithelium. Moreover, the cells also differed in the localization of their nuclei; the nuclei of anti-Calbindin labeled cells were found to be closer to the apical end of the cells while those of anti-GFSKLYFamide labeled cells were closer to the basal end. The results obtained through these studies will provide important information on echinoderm anatomy, nervous system connectivity and evolution, and for future studies of cellular differentiation during intestinal regeneration.

Disclosures: M.A. Lefebre: None. **J.E. Garcia-Arraras:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.22/AA9

Topic: E.04. Autonomic Regulation

Support: NIH Grant NS22189

NIH Grant EB018783

NIH Grant HD36020

NIH Grant NS061823

Craig H. Neilsen Foundation Award 124953

Title: Spinal transection alters dependence of void size on intra-burst EMG timing during spontaneous voiding in unanesthetized freely-moving rats

Authors: *J. S. CARP[1,2], B. K. LAPALLO[1], X. CHEN[1,2], J. R. WOLPAW[1,2,3];
[1]Lab. Neural Injury and Repair, Wadsworth Ctr, NY State Dept Hlth., Albany, NY; [2]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY; [3]Dept. Neurol., Stratton VA Med. Ctr., Albany, NY

Abstract: During voiding in rats, the external urethral sphincter (EUS) exhibits “bursting,” a phasic pattern of short periods of activity alternating with periods of inactivity (silent periods (SPs)). Urine flow during bursting is thought to occur during the SPs. In anesthetized rats, spinal cord transection (TX) decreases SP duration and voiding efficiency. The contribution of the SP to spontaneous voiding in unanesthetized rats is unknown. In the present study, we recorded EUS

electromyographic activity (EMG) and urine output in unanesthetized rats to assess the contribution of SP duration to void size during spontaneous micturition in freely-moving rats before and after TX. Female Sprague-Dawley rats (n=22) were implanted with fine stainless steel wires running subcutaneously from a skull-mounted pedestal to the dorsal surface of the pubic bone, where the free wire ends were secured by sutures adjacent to the ventral aspect of the EUS muscle. Rats were housed in metabolic cages for 24-hr epochs while EUS EMG (conducted by a flexible tether to a commutator) and voided urine weight (measured by force transducer) were recorded. After 2-8 weeks of recordings (Intact), 14 rats received mid-thoracic TX (MidT-TX; T8-T9; n=7) or thoraco-lumbar TX (TL-TX; T12-L2; n=7) and recordings were repeated over 4 weeks. Bursting was variably expressed in Intact rats, such that SPs could only be quantified in 17 of 22 animals. Bursting was strongly and consistently expressed after TX; SPs were quantified in all 14 TX rats. SP decreased after TX (mean SP±SE= 121±10 ms, 60±9 ms, and 68±10 ms for Intact, TL-TX, and MidT-TX rats, respectively). In Intact rats, void size was positively correlated with SP on a void-by-void basis. After TX at either level there was no correlation between void size and SP. Void size was significantly larger in TX rats than in Intact rats when SP was restricted to a matched narrow range of short SP values. No differences were found in SP between the two TX levels. Total bursting time was significantly longer in TX rats than in Intact rats; a significant positive correlation was found between void size and cumulative SP in Intact, MidT-TX, and TL-TX rats. The bursting efficacy (void size/duration of bursting) decreased after TX at either level. These results suggest that during spontaneous voiding in rats with intact neuraxes, intermittent EUS relaxation during bursting facilitates urine production. After spinal TX, bursting is still important for voiding, but the contribution of the SP is reduced due to a TX-induced decrease in the intrinsic ability of bursting to void urine.

Disclosures: **J.S. Carp:** None. **B.K. LaPallo:** None. **X. Chen:** None. **J.R. Wolpaw:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.23/AA10

Topic: E.04. Autonomic Regulation

Support: GSK

Title: Pudendal nerve stimulation restores bladder capacity in rat prostaglandin E2 overactive bladder model

Authors: ***J. A. HOKANSON**[1], C. LANGDALE[2], A. SRIDHAR[3], W. GRILL[2];
[1]Bioengineering, Duke University, Dept Biomed. Engin., Durham, NC; [2]Biomed. Engin., Duke Univ., Durham, NC; [3]GlaxoSmithKline, Stevenage, United Kingdom

Abstract: Overactive bladder (OAB), resulting in urgency, frequency, and incontinence, is a highly prevalent condition that leads to medical complications and decreased quality of life. Treatments for OAB include behavioral therapy, exercise therapy, pharmacotherapy, and electrical stimulation. Behavioral and exercise therapy have limited efficacy, and pharmacotherapy has dose-limiting side effects. The Medtronic Interstim device targets the S3 sacral spinal nerve, but is considered relatively non-selective. Electrical stimulation of peripheral nerves may provide greater selectivity thereby increasing efficacy and reducing side effects. We hypothesized that electrical stimulation of the "sensory" branch of the pudendal (SBPudN) nerve would restore bladder capacity (BC) and voiding efficiency (VE) in the rat prostaglandin E2 (PGE2) model of OAB. The aims of this study were to quantify functional changes (BC and VE) after intravesical installation of PGE2 and to measure the effects of SBPudN stimulation on BC and VE. Acute experiments were conducted in urethane (1.2 g/kg, SC) anesthetized female Wistar rats using *in vivo* cystometry (CMG). A PE-90 catheter was placed to measure bladder pressure and for intravesical infusion of saline and PGE2 (100 μM). Bipolar electrodes were placed on the external urethral sphincter (EUS) to measure electromyographic (EMG) activity and a bipolar nerve cuff was placed on the SBPudN for stimulation. In a subset of trials after PGE2 infusion, continuous SBPudN stimulation was delivered at different amplitudes (0.5 - 10x EUS reflex threshold) and frequencies (1, 10, 20, and 30 Hz). With rats prone with their abdomens lying on a heating pad, PGE2 administration quickly (within 50 minutes, on average) disrupted coordinated contraction of the bladder, decreased BC (53% of original, on average), and led to overflow incontinence. With rats in a supine position (abdomen closed), with a heating pad on their backs, PGE2 infusion decreased BC (62% of original) and increased VE (210% of original), but did not disrupt coordinated bladder contraction or cause overflow incontinence. Additional testing in the prone position with a heating pad on their backs confirmed that the overflow incontinence was likely due to increased heat delivered to the abdomen and/or catheter during PGE2 administration. In both prone and supine positons, SBPudN stimulation returned BC to control levels (105% of original). Stimulation decreased VE relative to the PGE2 condition (65% relative to PGE2), but still was higher than in the control case (138% of original). These findings suggest that SBPudN stimulation is a promising approach for treatment of OAB.

Disclosures: J.A. Hokanson: None. **C. Langdale:** None. **A. Sridhar:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **W. Grill:** None.

Poster

348. Gastrointestinal, Renal/Urinary, and Reproductive Regulation

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 348.24/AA11

Topic: E.04. Autonomic Regulation

Support: NIH Grant DK051369-16S1

NIH Grant DK060481

Title: Mechanism(s) of transforming growth factor-beta (TGF-β) mediated bladder afferent nerve hyperexcitability

Authors: *E. J. GONZALEZ, M. A. VIZZARD;
Neurolog. Sci., Univ. of Vermont, Burlington, VT

Abstract: The neural circuitry underlying the micturition reflex is often compromised following neural diseases, injuries and inflammatory conditions. We have previously demonstrated that TGF-β1 contributes to afferent nerve hyperexcitability in an experimental cystitis model of Bladder Pain Syndrome (BPS)/Interstitial Cystitis (IC). We hypothesize that afferent hyperexcitability may result from urothelial cells secreting neuroactive molecules, including adenosine triphosphate (ATP), that signal to the suburothelial nervous network. We used whole bladder preparations (n=4-8) isolated from C57BL6 mice (3-6 month old, male) to determine the role of TGF-β1 in urothelial mucosal ATP release. Intravesical instillation of recombinant TGF-β1 (10 ng/ml) significantly ($p \leq 0.01$) increased mucosal ATP release. The release of ATP was attenuated with the co-administration of a TβR-1 inhibitor, SB505124 (5 μM), suggesting the response was specific to TGF-β1 instillation. TGF-β-mediated ATP release was also reduced by either carbenoxolone (100 μM) or brefeldin A (10 μM) suggesting TGF-β1 stimulates ATP release via hemichannel and vesicular mechanisms. Taken together, these results demonstrate a role for TGF-β in urothelial signaling that may contribute to afferent nerve hyperexcitability and underlie peripheral and central sensitization. Targeting TGF-β within the sensory components of the micturition reflex may be a therapeutic approach to improve bladder function.

Disclosures: E.J. Gonzalez: None. **M.A. Vizzard:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.01/AA12

Topic: F.02. Animal Cognition and Behavior

Support: Israel Science Foundation 663/13

Title: Differences in the mechanisms of extinction and medial prefrontal cortex plasticity in the adult and post-weanling animals

Authors: *M. MAROUN[1], R. SCHAYEK[2];
[2]Sagol Dept. of Neurobio., [1]Univ. of Haifa, Haifa, Israel

Abstract: The neural circuit linking the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) is a part of the limbic system known to mediate emotional learning, and it has crucial roles in both the acquisition and the extinction of fear conditioning. Exposure to behavioral stress is a major factor known to impair extinction, enhance fear responses and to severely affect memory processes. Exposure to behavioral stress is widely assumed as a major factor to enhance the development of anxiety disorders.We recently addressed the effects of exposure to stress in a form of exposure of 30 minutes to the elevated platform, on extinction and plasticity in the mPFC in the post-weanling pup. (Schayek and Maroun, Biological Psychiatry, 2014. Surprisingly, our results show that exposure to stress is associated with enhanced ability to extinguish fear responses in the post-weanling pup. Similarly, stress was associated with enhanced LTP in the mPFC. When recording in the BLA, stress results in enhanced LTP in the adult animal without leading to any potentiation in the post-weanling pup.Furthermore, we also show that systemic injections of the D1 receptors agonist, SKF38393 enhanced BLA-LTP in the post-weanling animal without affecting the induction of LTP in the adult animal. These results show that stress deferentially modulates extinction and plasticity in the post-weanling pup as compared to the adult animal and point that the mechanisms of extinction and stress-induced changes in extinction and plasticity are not similar across development.

Disclosures: M. Maroun: None. **R. Schayek:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.02/AA13

Topic: E.05. Stress and the Brain

Support: ESRC Grant ES/K005723/1

Title: Autobiographical memory functioning in maltreated children and the theory of Latent Vulnerability

Authors: *V. PUETZ[1], E. VIDING[1], A. PALMER[1], P. KELLY[1], E. A. MAGUIRE[2], A. MECHELLI[3], E. MCCRORY[1];
[1]Dept. of Psychology and Language Sci., Univ. Col. London, London, United Kingdom; [2]Inst. of Neurol., Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom; [3]Dept. of Psychosis Studies, Inst. of Psychiatry, Psychology & Neuroscience, King's Col. London, London, United Kingdom

Abstract: Objective: Childhood maltreatment has been associated with alterations in autobiographical memory (ABM) functioning. A pattern of over-general ABM has also been associated with child psychopathology, and may represent a cognitive risk factor for psychiatric

disorder. The current study aimed to investigate the neural systems supporting ABM in children exposed to maltreatment and matched controls. Methods: 34 children with a documented history of maltreatment (age=12.53±1.6; n=17 boys) and 33 control children (age=12.66±1.3; n=15 boys) matched on age, gender, IQ, SES, ethnicity and pubertal status were recruited. Participants underwent fMRI, recalling ABMs in response to positively and negatively emotionally valenced cue words. Whole-brain and a-priori ROI analyses were conducted to investigate differential neural activation between the groups (Monte-Carlo-corrected at p .05). All participants and their caregivers gave written informed assent and consent respectively. Results: Greater valence-dependent neural differences in the control group to positive relative to negative stimuli were observed in the bilateral hippocampus, comparable to previous findings reported for adults. Between-group comparisons indicated significantly heightened neural activation in the right hippocampus in children exposed to maltreatment relative to controls, during ABM recall to negative versus positive cues. Post-task debriefing indicated that memories were comparable in terms of difficulty to recall, vividness and remoteness (all $p>0.1$). Conclusions: In line with previous research in adults, control children showed robust activations in memory and arousal related brain areas in response to positive autobiographical memory recall. The significantly heightened activity in the right hippocampus during negative recall may indicate greater salience of negative personal memories in children exposed to maltreatment, a pattern consistent with a cognitive strategy of functional avoidance. According to the theory of Latent Vulnerability (McCrory & Viding, in press), childhood maltreatment calibrates and sensitizes a number of neurocognitive systems in line with early at-risk environments, with potentially adaptive benefits in the short term but with longer-term costs. It is suggested that ABM functioning may represent a neurocognitive system that is altered as a consequence of maltreatment exposure, embedding latent vulnerability to future stressors, thereby increasing risk of later psychiatric disorder.

Disclosures: V. Puetz: None. **E. Viding:** None. **A. Palmer:** None. **P. Kelly:** None. **E.A. Maguire:** None. **A. Mechelli:** None. **E. McCrory:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.03/AA14

Topic: E.05. Stress and the Brain

Title: Implications of maternal stress during pregnancy for neonatal amygdala connectivity and development of fear

Authors: *A. GRAHAM[1], D. A. FAIR[1], J. RASMUSSEN[2], M. RUDOLPH[1], J. H. GILMORE[3], M. A. STYNER[3], S. ENTRINGER[4,2], P. WADHWA[2], C. BUSS[4,2];

[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Univ. of California, Irvine, Irvine, CA; [3]UNC, Chapel Hill, NC; [4]Charité Univ. of Med., Berlin, Germany

Abstract: Human infants exhibit multiple fear-related behaviors beginning in the first year of life. Animal models indicate that prenatal stress shapes development of fear via effects on underlying amygdala circuitry. However, the influence of prenatal stress on brain systems involved in the emergence of fear in humans remains poorly understood. Moreover, despite the understanding that emotion and cognition are inextricably linked at the level of brain and behavior, development of fear during infancy is not often considered in the context of cognitive development. This is important because the implications of early fearfulness for long-term outcomes likely depend on cognitive development. Data from an ongoing prospective longitudinal study of maternal-fetal/infant dyads (N=36 infants;15 females) were analyzed. Ambulatory momentary assessments of maternal stress were conducted in each trimester of pregnancy over 4 days. Resting state functional connectivity of the amygdala was examined in neonates (M=25.8 days, SD=12.9). Infant fear (Infant Behavior Questionnaire) and cognitive development (Bayley Scales of Infant Development) were assessed at 6-months-of-age (M=196 days, SD=13.5). Stronger, positive amygdala connectivity to anterior insula, ventral striatum (VS), parahippocampal gyrus, premotor cortex and temporoparietal junction predicted higher fear. Stronger amygdala connectivity to anterior medial prefrontal cortex (aMPFC) predicted a specific phenotype of higher fear and advanced cognitive development. This highlights the importance of considering fear in the context of cognition, and may shed light on a pattern of early amygdala connectivity that facilitates advanced emotional development balanced by parallel cognitive development. We then examined maternal stress during pregnancy as a predictor of these connectivity patterns. An increase in stress from early to late pregnancy (adjusting for stress in early pregnancy) predicted weaker amygdala-VS (β=-.522, p=.000) and amygdala-aMPFC (β=-.546, p=.000) connectivity in neonates. These findings suggest that maternal stress during pregnancy is associated with “slowed” development of these emotion-cognition circuits. Amygdala functional connections evident by the time of birth predict the early emergence of specific fear-cognition phenotypes. These functional connections are in turn influenced by levels of maternal stress during pregnancy. The findings highlight that high fear is not always associated with reduced cognitive skills, particularly in this early developmental period. The neural underpinnings of fear should be considered in the context of cognitive domains.

Disclosures: A. Graham: None. **D.A. Fair:** None. **J. Rasmussen:** None. **M. Rudolph:** None. **J.H. Gilmore:** None. **M.A. Styner:** None. **S. Entringer:** None. **P. Wadhwa:** None. **C. Buss:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.04/AA15

Topic: E.05. Stress and the Brain

Support: NIH Grant R01DA033369

NIH Grant R01DA031579

NIH Grant P30DA023026

NIH Grant T32HD0737625

Title: Early life stress is associated with decreased reward-related activity and intrinsic functional connectivity of the ventral striatum as well as symptoms of depression in adulthood

Authors: *J. L. HANSON[1,3], A. KNODT[1], K. A. DODGE[1,2], A. R. HARIRI[1];
[1]Psychology & Neurosci., [2]Publ. Policy, Duke Univ., Durham, NC; [3]Ctr. for Developmental Sci., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Studies of neural mechanisms through which early life stress (ELS) is associated with later psychopathology including depression have focused on dysfunction of a corticolimbic circuit supporting threat processing and stress responsiveness. However, emerging research further implicates dysfunction of a corticostriatal circuit supporting motivation and reward learning in stress-related psychopathology including depression. Here, we present results from two complementary studies examining associations between ELS, reward-related corticostriatal circuit function, and symptoms of depression. First, using a simple card-guessing task and fMRI, we examined reward-related activity of the ventral striatum (VS) in relation to ELS in a cohort of adults followed continuously since kindergarten (n=72). We found that greater levels of ELS during childhood and adolescence were related to lower reward-related VS activity in adulthood (Right VS, β=-0.248, p=0.03). Extending these results, we then examined the relation between ELS and intrinsic connectivity of the VS derived from resting-state fMRI in a large independent cohort of adults (n=598). We found that higher ELS was related to increased negative connectivity between the right VS and ventromedial prefrontal cortex (β=-0.202, p<.005). Moreover, individual differences in this intrinsic connectivity were associated with self-reported symptoms of depression (β=-0.13, p<.005). These converging patterns suggest that altered corticostriatal circuit activity and intrinsic connectivity represent mechanisms through which ELS can affect the later emergence of psychopathology.

Disclosures: J.L. Hanson: None. **A. Knodt:** None. **K.A. Dodge:** None. **A.R. Hariri:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.05/AA16

Topic: E.05. Stress and the Brain

Support: R01MH070890-09A1

R21NS088975-01

R03DA036645-01A1

P01DA022446

Title: Development of dopamine functional pathways during infancy: normal trajectory and disruptions due to genetic and environmental risks

Authors: A. SALZWEDEL, W. LIN, K. GREWEN, J. GILMORE, *W. GAO;
Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Dopamine (DA) is expressed prior to synaptogenesis and DA-mediated signaling plays a fundamental neurodevelopmental role in the brain's initial functional circuit formation. Therefore, delineation of the normal developmental time-line of the DA systems during early infancy_when the brain is dynamically developing, plastic, and susceptible to disruptions induced by either genetic or environmental risk factors_is crucial for both improved scientific understanding and better clinical intervention. In this study, the developmental time-lines of two fundamental DA pathways originated from the ventral tegmental area (VTA) and substantia nigra (SN) were delineated during infancy. A large sample of healthy infants underwent resting-state connectivity fMRI; 234 (118 Females) neonates, 150 (71 F) one-year olds, and 116 (56 F) two-year olds. Seed-regions were based on an a priori parcellation of the VTA and SN warped into infant space. Genetic and environmental influences on DA-pathway connectivity were characterized in infants at high risk of developing schizophrenia (i.e., mothers diagnosed with schizophrenia; 14 (8 F) neonate, 15 (8 F) one year olds, 16 (7 F) two-year olds) and in neonates with prenatal drug exposure (68 (33 F) healthy controls, 32 (16 F) with poly-drug exposure [NCOC, i.e., nicotine, alcohol, marijuana, etc] and 42 (22 F) with poly-drug exposure + Cocaine [PCE]). In the normative sample, our results indicated that functional circuits anchored by the VTA and SN are already differentiated at birth with the VTA circuit extending into ventromedial striatum and SN connectivity projecting to dorsal striatum and motor-related central parietal regions. With development, the SN connectivity remains largely constant but the VTA circuit shows extension into medial prefrontal areas in both 1- and 2-year olds. Compared with normal controls, infants at high risk for schizophrenia (HR) show significantly more decline of SN-motor connectivity and more enhancement of VTA-cortical-area connectivity with age. Finally, neonates with prenatal drug exposures (both NCOC and PCE) show region-specific disruption profiles (4 significant clusters detected) within both the VTA and SN circuits. Our results indicate that the SN-centered DA pathway mature earlier than the VTA however the VTA circuit shows more substantial postnatal development in normal infants. Importantly, this normal developmental process is disrupted by both genetic risks for schizophrenia and environmental risks from prenatal drug exposure as early as in the neonatal period. These results call for more attention and potentially early intervention to these at-risk infants.

Disclosures: **A. Salzwedel:** None. **W. Lin:** None. **K. Grewen:** None. **J. Gilmore:** None. **W. Gao:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.07/AA17

Topic: E.05. Stress and the Brain

Support: Simches Family to SLA

NARSAD to JLL

Title: Effects of early life stress on amygdala, prefrontal cortex, and raphe GABA levels and its relatation to depressive-like behavior

Authors: ***S. L. ANDERSEN**[1], J. L. LUKKES[1], S. MEDA[2], B. THOMPSON[2], N. FREUND[3]; [1]Dept Psychiatry, McLean Hosp, Belmont, MA; [2]McLean Hosp., Belmont, MA; [3]Univ. of Tubingen, Tubingen, Germany

Abstract: Clinical and preclinical studies show that early life stress (ELS) is associated with greater affective dysfunction. In depression-vulnerable subjects with a history of ELS, imaging studies reveal that the ventral medial prefrontal cortex (vmPFC) can only transiently inhibit amygdala activation to repeated stress and then amygdala activity is elevated; depression-resilient individuals show greater vmPFC activity and less amygdala activity in response to stress. Increased amygdala responses to an acute stress in ELS may be due to low GABA inhibition or decreased regulation by the mPFC, or both. Here, we wanted to determine the relationships between ELS, GABA (e.g., parvalbumin cells; PVB), and depressive behavior, and whether behavior and PVB changes are preventable with the anti-inflammatory agent, COX-2. Separation of rat pups from the mother leads to a cascade of maladaptive behaviors, including depressive-like behavior. Sprague-Dawley female rats were exposed to ELS between 2 and 20 days of age or raised under typical animal facility rearing conditions (AFR). Subjects were tested for helplessness during adolescence. The effects of an acute stressor of witnessing their peers in distress for ~2 hours the day before testing were also determined. Latency to escape footshock and escape failures were higher in ELS than AFR subjects, as we have shown previously. The second stressor elevated latency to escape in AFR controls, but reduced latency in ELS subjects. These data suggest that ELS subjects may be better able to cope with an acute stressor than AFR subjects, but overall, their functioning is worse than AFR subjects. COX-2 inhibition prevented depressive effects in ELS subjects. Regional brain dissections coupled with Western immuoblot for PVB protein allowed us to specifically relate mPFC PVB changes to depressive-like behavior. The basolateral amygdala (BLA) and the dorsal raphe nucleus are involved in

regulating emotion and depression. PVB decreased in the mPFC and the dorsal raphe nucleus, but not the BLA in ELS relative to AFR subjects. Exposure to an acute stressor before testing, however, reduced PVB levels in the BLA and increased PFC levels to AFR control levels. In addition, we show for the first time that PFC levels of PVB inversely correlate with the latency to escape a footshock. These data suggest that elevated reactivity of the amygdala in individuals with a history of ELS may be partially mediated by reduced GABAergic inhibition that leads to increased activity when provoked. Finally, an acute stressor in ELS rats is also able to increase PVB levels, which may further explain a dampening down of mPFC function in these individuals.

Disclosures: S.L. Andersen: None. **J.L. Lukkes:** None. **S. Meda:** None. **B. Thompson:** None. **N. Freund:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.08/AA18

Topic: E.05. Stress and the Brain

Support: K23MH105179

R01 HD05709801

P30 HD062171

K02 NS089852

McDonnell Center for Systems Neuroscience

Title: Impact of preterm birth on structural and functional connectivity in neonates

Authors: *C. ROGERS[1], C. HERZMANN[1], T. SMYSER[1], J. SHIMONY[1], J. ACKERMAN, Jr.[1], J. NEIL[2], C. SMYSER[1];
[1]Washington Univ. Sch. of Med., Saint Louis, MO; [2]Boston Children's Hosp., Boston, MA

Abstract: Premature birth is a deleterious early life stressor associated with altered cerebral gray matter and white matter development. It is also associated with developmental deficits and increased rates of psychopathology including ADHD, Autism Spectrum Disorder and anxiety disorders. There is increasing evidence these developmental deficits may be subsequent to altered cerebral development, particularly in those children born at the earliest gestational ages. However, few studies have applied multimodal imaging techniques to concomitantly assess structural and functional connectivity in this population. This study compared structural and functional connectivity in very preterm infants (VPT; gestational age<30 weeks; N=76) without

significant brain injury to that of healthy, full term infants (FT; gestational age≥37 weeks; N=58). All infants underwent MRI at term-equivalent age (36-42 weeks postmenstrual age), including acquisition of diffusion tensor imaging (DTI) and resting state fMRI (rs-fMRI) sequences. DTI data was analyzed using tract based spatial statistics (TBSS), assessing group differences in fractional anisotropy (FA). rs-fMRI data was analyzed to determine measures of correlation and covariance within and between seven canonical resting state networks (RSNs). TBSS results demonstrated widespread reductions in white matter tract FA in VPT versus FT infants (Figure 1A). VPT infants also demonstrated reduced correlation and covariance within and between all RSNs (Figure 1B). Among RSNs involved in attentional, social-communicative and affective processing, the default mode (DMN; p=.001) and frontoparietal (FPC; p<.001) networks were notably affected. White matter tracts connecting hubs of these RSNs (*e.g.,* cingulum, anterior limb internal capsule) were among those demonstrating greater between group differences. These results suggest preterm birth causes widespread disruption of functional and structural connectivity of the developing brain, including regions linked to the psychiatric disorders that occur commonly in preterm children.

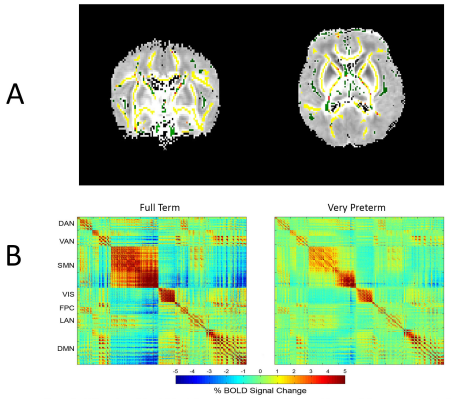


Figure 1. A) Tract Based Spatial Statistics analysis comparing Term and Preterm infants at term equivalent. Tracts are overlaid on FA maps of the neonates. Red and yellow note regions of significantly lower FA in preterm infants. B) Group mean covariance matrices generated using 1065 cortical gray matter regions of interest. The block structure corresponds to RSNs to which each region is assigned, including the dorsal attention (DAN), ventral attention (VAN), somatomotor (SMN), visual (VIS), frontoparietal control (FPC), language (LAN) and default mode (DMN) networks. (A) Term infants; (B) Preterm infants at term equivalent PMA. Note similarity of block structure in (A) and (B). This similarity reflects down-scaling of positive and negative rs-fMRI covariance values in the preterm subjects relative to the term subjects.

Disclosures: **C. Rogers:** None. **C. Herzmann:** None. **T. Smyser:** None. **J. Shimony:** None. **J. Ackerman:** None. **J. Neil:** None. **C. Smyser:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.09/AA19

Topic: E.05. Stress and the Brain

Support: NIH Grant P01-AG020166

NIH Grant F32HD078048

NIH Grant R01 HD058502

NIH Grant R01 AG018436

NIH Grant P30 DA027827

Title: Frontal EEG asymmetry and childhood maltreatment history predict systemic inflammation in middle-aged adults

Authors: *C. E. HOSTINAR[1], R. J. DAVIDSON[2], T. E. SEEMAN[3], D. K. MROCZEK[1], M. E. LACHMAN[4], G. E. MILLER[1];
[1]Northwestern Univ., Evanston, IL; [2]Univ. of Wisconsin, Madison, WI; [3]UCLA, Los Angeles, CA; [4]Brandeis Univ., Waltham, MA

Abstract: Frontal EEG asymmetry is thought to reflect temperamental variations in affective style, with studies showing that greater right frontal activity at rest predicts enhanced emotional responding to threatening or negative stimuli. A diathesis-stress model has been proposed to explain how this neuro-affective style might predispose to psychopathology, such that greater right frontal activity would constitute a vulnerability factor under stressful conditions, but would not predict symptomatology in the absence of circumstances that elicit an emotional response. Much less is known about the extent to which greater right frontal activity might also serve as a diathesis for physical illness in individuals exposed to an environmental stressor that elicits negative emotions. The present study examined the hypothesis that greater resting right frontal EEG activity might exacerbate the effects of childhood maltreatment on levels of adult systemic low-grade inflammation, a risk factor for several chronic diseases of aging (e.g., coronary heart disease). Resting EEG, serum inflammatory biomarkers, and self-report psychological measures were drawn from a group of 313 middle-aged adults from the national MIDUS study. Log alpha power in the right hemisphere was subtracted from log alpha power in the left hemisphere to create an index of laterality, considering both the lower (8-10 Hz) and upper (10-13 Hz) ranges of the alpha band. A composite measure of inflammation was derived from five biomarkers, including serum levels of interleukin-6, C-reactive protein, fibrinogen, E-selectin, and ICAM-1. Higher levels of right frontal neural activity and of childhood maltreatment exposure both independently predicted greater systemic inflammation ($\beta = .12$, $p = .03$ and $\beta = .11$, $p = .04$, respectively). There was also a significant interaction ($\beta = .16$, $p = .005$) such that individuals who had both greater right frontal activity and exposure to more types of maltreatment exhibited the greatest levels of inflammation. The interaction remained significant after adjustment for demographic and biomedical covariates. These results suggest that greater right frontal neural activity might amplify the health risks associated with a history of childhood maltreatment.

Disclosures: C.E. Hostinar: None. **R.J. Davidson:** None. **T.E. Seeman:** None. **D.K. Mroczek:** None. **M.E. Lachman:** None. **G.E. Miller:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.10/AA20

Topic: E.05. Stress and the Brain

Support: NIH Grant P50 MH078105

NIH Grant F31 MH086203

National Center for Research Resources Grant P51RR165

Office of Research Infrastructure Programs/OD Grant P51OD11132

NIH Grant P50 MH078105-S1

NIH Grant R21 HD055255

NIH Grant R01 MH091645

Title: Infant maltreatment alters limbic white matter tracts: support for the match/mismatch hypothesis?

Authors: *B. R. HOWELL[1], M. AHN[2], J. R. GODFREY[3], Y. SHI[5], G. NAIR[7], X. HU[8], M. A. STYNER[5,6], M. M. SANCHEZ[3,4];
[1]Inst. of Child Develop., Univ. of Minnesota, Minneapolis, MN; [2]Dept. of Mathematics and Statistics, Univ. of Nevada, Reno, NV; [3]Yerkes Natl. Primate Res. Ctr., [4]Psychiatry & Behavioral Sci., Emory Univ., Atlanta, GA; [5]Psychiatry, [6]Computer Sci., Univ. of North Carolina, Chapel Hill, NC; [7]Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD; [8]Biomed. Engin., Emory Univ. and Georgia Inst. of Technol., Atlant, GA

Abstract: Early social experiences, such as maternal care, are important for socioemotional development in primates. Victims of childhood maltreatment are at higher risk for psychopathology including depression and anxiety. These alterations in socioemotional behaviors are related to neurobiological alterations in specific brain circuits. Utilizing a naturalistic nonhuman primate model of infant maltreatment we investigated how the neurodevelopmental effects of early experience unfold in white matter (WM) tracts involved in emotion and social behavior. Our model includes comorbid physical abuse and neglect during infancy, often interspersed with competent care much like in humans. In previous studies using this model we have reported long-term effects on social behavior and emotional reactivity, as well as alterations in WM integrity. The current study expands these findings by following a cohort of rhesus monkeys randomly assigned at birth to either competent or maltreating maternal care longitudinally, from 2 weeks through 18 months of age. Using this crossfostering design we are able to disentangle the effects of heritable factors from those of the experience itself. We used a longitudinal diffusion tensor imaging (DTI) rhesus brain atlas to gather DTI metrics (e.g. fractional anisotropy, FA, an indicator of structural integrity) along WM tracts and applied

sophisticated longitudinal statistical models to examine the effects of experience versus heritable factors (and their interactions) throughout this developmental period. We found that FA in the inferior longitudinal fasciculus and the middle longitudinal fasciculus, long association tracts involved in visual processing and possibly auditory processing, respectively, with both playing a role in socioemotional behavior, were affected by experience, but also influenced by heritable factors. Animals born to competent mothers and fostered to competent mothers had significantly higher FA than those in the 3 other foster groups. These results may support the environmental match/mismatch hypothesis that suggests that early life adversity leads to changes that are adaptive to the challenging environment, and that these adaptions may be passed from one generation to the next to prepare offspring to adapt to that environment. Another possibility is that there is a cumulative effect of maltreatment experience and the heritable factors passed from maltreating mothers to their offspring. Ongoing studies are aimed at better defining success within each environmental context and at integrating the current neurobiological findings with behavioral outcomes in the crossfostered offspring.

Disclosures: **B.R. Howell:** None. **M. Ahn:** None. **J.R. Godfrey:** None. **Y. Shi:** None. **G. Nair:** None. **X. Hu:** None. **M.A. Styner:** None. **M.M. Sanchez:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.11/AA21

Topic: E.05. Stress and the Brain

Support: Klaus J. Jacobs Foundation Research Prize to Dante Cicchetti

Imaging support from the Rochester Center for Brain Imaging

Pilot grant from the College of Arts, Science and Engineering, University of Rochester

Title: Corticolimbic activation and functional connectivity in adults who experienced child maltreatment

Authors: *K. JEDD[1], R. H. HUNT[1], D. CICCHETTI[1], E. HUNT[2], F. ROGOSCH[2], S. TOTH[2], R. A. COWELL[3], K. M. THOMAS[1];
[1]Inst. of Child Develop., Univ. of Minnesota, Minneapolis, MN; [2]Univ. of Rochester, Rochester, Minnesota, NY; [3]St. Norbert Col., De Pere, WI

Abstract: Introduction. Child maltreatment is related to a host of problems with emotion perception and regulation, often manifesting as depression, conduct disorder, and substance abuse (Cicchetti & Valentino, 2006; DeBellis, 2001). Children with a history of maltreatment show altered emotion perception and emotional processing (Pollak & Tolley-Schell, 2003, Pollak

et al., 2000, Cicchetti & Curtis, 2005). Limbic structures in the brain may be especially sensitive to early life stress (Tottenham, 2012). The few functional studies that exist with maltreated samples point to hyperactivity in the amygdala following childhood maltreatment (e.g. Dannlowski et al., 2012). However, little is known about the lasting impact of childhood maltreatment on functional connectivity of the limbic system in adulthood. **Methods**. Participants included 71 adults from high-risk, low socio-economic backgrounds. 33 participants experienced documented cases of child maltreatment including emotional maltreatment, physical neglect, physical abuse, and/or sexual abuse; 38 participants had no history of maltreatment. All participants were recruited from a longitudinal sample of children who participated in a summer camp research program designed for low-income children. In the scanner, participants were asked to match a target face with one of two other faces based on emotional expression (Hariri et al., 1999). Whole brain analyses were conducted to examine group differences in task activation. In addition, a psychophysiological interaction (PPI) analysis assessed group differences in task based functional connectivity using a bilateral amygdala seed. **Results.** The maltreated group had greater activation than the comparison group in bilateral putamen/caudate and in right dorsolateral prefrontal cortex (dlPFC). Later onset of maltreatment was associated with increased activity in right dlPFC. Additionally, participants who experienced maltreatment in fewer developmental periods had greater activity in right dlPFC, left nucleus accumbens/putamen, and right amygdala. In the PPI analysis, the maltreated group showed increased connectivity of the amygdala with left hippocampus, bilateral parahippocampal gyrus, dorsal medial PFC, right inferior frontal gyrus, and right dlPFC. **Discussion.** These results indicate that childhood maltreatment is associated with lasting functional alterations in limbic circuitry. Altered neural processing in the limbic system could be a contributing factor in the development of psychopathology, however more research is necessary to understand how limbic structures respond to the intense stress of childhood maltreatment.

Disclosures: **K. Jedd:** None. **R.H. Hunt:** None. **D. Cicchetti:** None. **E. Hunt:** None. **F. Rogosch:** None. **S. Toth:** None. **R.A. Cowell:** None. **K.M. Thomas:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.12/AA22

Topic: E.05. Stress and the Brain

Support: NIH-MH091451

NIH-DC009910

T32MH096331

Title: Rescue of neurobehavioral deficits following infant abuse: The role of maternal odor

Authors: *R. E. PERRY, R. M. SULLIVAN;
Emotional Brain Inst., Nathan Kline Inst., Orangeburg, NY

Abstract: Experiencing infant abuse alters the expression of fear and aggression throughout development, with a heightened sensitivity to threat and an increased incidence of aggressive behaviors emerging by adolescence in humans. Here we use a rodent model of infant abuse to assess the expression of fear and aggression throughout the lifespan, explore possible modes of rescuing the neurobehavioral deficits that emerge following abuse, and investigate the neurobiological underpinnings mediating the effects of abuse. Infant rat pups were raised with a normal or abusive mother from postnatal days (PN) 8-12, and tested in a novel Threat Response Selection Test (TRST) at post-weaning (PN26-30), adolescence (PN40-45), and adulthood. In the TRST, individuals were placed in the center of a tri-sectioned arena and given the option to approach a predator odor (fox urine), or hide in a provided hut. Active (i.e. rearing, exploration) and passive (i.e. freezing, hiding) defensive responses were scored. Additionally, aggression was assessed in a Resident Intruder Test (RIT). In the RIT, animals were single housed for a week, before introducing a smaller, same sex intruder into their home cage for 10 minutes. Offensive and defensive behaviors were assessed. Lastly, the effects of infusing maternal odor into the testing arenas during the TRST and RIT were assessed on the adult response to threat following abuse. Activation of the prefrontal cortex (PFC), amygdala, hypothalamus, and periacqueductal gray (PAG) was scored following the TRST, via assessment of c-Fos activity. Post-weaning rats that experienced infant abuse showed heightened avoidance of fox urine and increased hiding in the provided hut in the TRST. However, in adulthood, previously abused rats displayed decreased hiding, increased approach and interaction with the predator odor, and decreased amygdala activation, relative to typically-reared rats. Additionally, infant abused rats showed increased offensive behaviors in the RIT, including lateral/dorsal attacks, pinning, boxing, and chasing the intruder. Importantly, the presentation of maternal odor at the time of testing for the RIT and TRST normalized the behavior of previously abused rats in adulthood, seemingly through a circuit involving the prefrontal cortex. Infant abuse alters responses to threatening stimuli throughout development, and decreases amygdala activation to threat by adulthood, making it more likely for one to place itself in harm's way. Understanding the neurobiology of threat responding and its modulation by early-life experience will provide insight for treatment of fear and aggression-related disorders that occur after abuse.

Disclosures: R.E. Perry: None. **R.M. Sullivan:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.13/AA23

Topic: E.05. Stress and the Brain

Support: APF COGDOP Award

APF Koppitz Fellowship

NIH Grant R01MH091864

NSF Graduate Research Fellowship

Title: Experimental manipulation of prefrontal cortex differentially affects amygdala reactivity and connectivity following early-life stress

Authors: *D. G. GEE[1], B. GOFF[2], L. GABARD-DURNAM[3], C. CALDERA[2], D. FARERI[3], D. LUMIAN[4], J. FLANNERY[5], N. TOTTENHAM[3];
[1]Psychology Dept, Weill Cornell Med. Col., New York, NY; [2]UCLA, Los Angeles, CA; [3]Columbia Univ., New York, NY; [4]Univ. of Denver, Denver, CO; [5]Univ. of Oregon, Eugene, OR

Abstract: Early-life stress can have profound and lasting effects on affective development and behavior. Humans rely on their caregivers longer than any other species, making parental deprivation one of the most potent stressors for an infant. Prior correlational studies suggest that parental deprivation alters the development of amygdala-prefrontal circuitry and related anxiety. However, the functional nature of these abnormalities is unknown. Experimental manipulation of prefrontal cortex (PFC) via cognitive load has been shown to have sustained and lasting effects on amygdala reactivity during adulthood (e.g., Wagner & Heatherton, 2012). The current study took advantage of PFC-mediated effects to investigate the nature of the amygdala-PFC relationship during development in children (8-11 years old) who experienced parental deprivation via previous institutionalization (PI) during infancy and typically developing comparison children (8-11 years old) and adolescents (12-17 years old). Participants completed a novel fMRI task that manipulates cognitive load on subsequent brain activation to emotional stimuli. Conditions of high cognitive load (relative to low cognitive load) induced inverse amygdala-medial PFC functional connectivity and reduced amygdala reactivity in comparison adolescents, but not comparison children, who showed positive amygdala-medial PFC functional connectivity and increased amygdala reactivity. PI children showed the more mature pattern of inverse amygdala-medial PFC functional connectivity, and despite heightened amygdala reactivity at baseline (low cognitive load), they also showed reduced amygdala reactivity during high cognitive load. Thus, the nature of amygdala-PFC recruitment and interactions differ qualitatively between typically developing and PI children, particularly under conditions that demand more cognitive resources. The finding that PI children resemble comparison adolescents suggests that early-life stress may accelerate amygdala-prefrontal development. Amygdala-prefrontal phenotypes following parental deprivation may be ontogenetic adaptations that the developing system makes in order to meet the demands of an adverse environment but are likely to have long-term consequences on emotional behavior.

Disclosures: D.G. Gee: None. **B. Goff:** None. **L. Gabard-Durnam:** None. **C. Caldera:** None. **D. Fareri:** None. **D. Lumian:** None. **J. Flannery:** None. **N. Tottenham:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.14/AA24

Topic: E.05. Stress and the Brain

Support: NIH RO1 MH065283

Title: Exercise induces age-dependent neuroplastic changes in limbic regions responsible for learning, memory and emotional behavior

Authors: *K. HULEN[1,2], A. MIKA[2,3], N. L. RUMIAN[2], A. K. HILLS[2], S. O. MCCONNELL[2], A. L. INGALLS-WILLIAMS[2], M. R. FLESHNER[2,3];
[1]Univ. of Colorado Boulder, Boulder, CO; [2]Dept. of Integrative Physiol., [3]Ctr. for Neurosci., Univ. of Colorado, Boulder, CO

Abstract: Voluntary wheel running exercise produces a milieu of adaptive plastic changes in rodent brains, including elevated levels of brain-derived neurotrophic factor (BDNF), increased transcription of synaptic proteins, epigenetic changes in genes involved in neuroplasticity, and structural changes such as increased spine and dendritic growth. These adaptations are abundant in the prefrontal cortex, amygdala, and hippocampus, which are regions involved in cognition and emotional behavior. These neuroplastic and behavioral enhancements occur rapidly after exercise onset; however, they are transient in adults and last only as long as exercise is continued. Interestingly, the brain is more plastic and sensitive to environmental influence during early life. Recent work from our lab, as well as others, demonstrates that exercise, when initiated during critical developmental periods, can produce neuroplastic changes that last well beyond exercise cessation. These previous studies, however, utilized exercise time courses that spanned multiple developmental stages. Therefore, the precise period during which exercise capitalizes on the plasticity of circuits that promote these long lasting changes is unclear. The current study tested exercise time courses that were brief enough to allow us to isolate and compare the impact of exercise on increases in plasticity proteins and neuronal structure within brain regions involved in cognition and behavior during discrete developmental periods. Male F344 rats (n=8 per group) at three distinct ages, juvenile (PND 24), adolescent (PND 40), and adult (PND 70), ran for one week, after which the wheels were immobilized and half of each cohort was sacrificed while the other half remained sedentary for two weeks. Brains were removed and processed for either *in situ* hybridization of mRNA for plasticity markers, or for Golgi staining of neuronal structure. Preliminary analyses demonstrated that increased levels of BDNF mRNA persist for 2 weeks after exercise cessation only when exercise was initiated during the juvenile period. Quantifications are currently in progress for dendritic complexity, spine density, and mRNA for plasticity proteins, including cyclic-AMP binding protein (CREB), tyrosine receptor

kinase B (TrkB), synapsin-I, and synaptophysin. These data suggest that exercise during critical periods may produce persistent improvements in neural plasticity and brain function.

Disclosures: **K. Hulen:** None. **A. Mika:** None. **N.L. Rumian:** None. **A.K. Hills:** None. **S.O. McConnell:** None. **A.L. Ingalls-Williams:** None. **M.R. Fleshner:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.15/AA25

Topic: E.05. Stress and the Brain

Support: NIH RO1 MH068283

Title: Early life exercise produces persistent alterations in serotonergic circuits and long-lasting stress resistance

Authors: ***A. MIKA**[1,2], N. L. RUMIAN[1], A. K. HILLS[1], S. O. MCCONNELL[1], C. A. BOUCHET[3], B. N. GREENWOOD[3], M. R. FLESHNER[1,2];
[1]Integrative Physiol., Univ. of Colorado, Boulder, Boulder, CO; [2]Ctr. for Neurosci., Univ. of Colorado, Boulder, CO; [3]Dept. of Psychology, Univ. of Colorado, Denver, CO

Abstract: Voluntary exercise protects against the anxiety and depressive-like behaviors produced by inescapable stress (IS), including exaggerated fear and deficits in shuttle box escape learning. When exercise is initiated in adulthood, however, the protective effects are transient and the organism is once again vulnerable to the behavioral consequences of IS shortly after exercise cessation. We have demonstrated that early life exercise produces lasting protection against IS-induced exaggerated fear and shuttle box escape deficits that persist despite termination of exercise. The underlying neural mechanisms for this persistent effect are unknown. In adults, the stress protective effects of exercise are dependent upon plastic changes within serotonergic (5-HT) circuits. Specifically, hyperactivation and sensitization of 5-HT neurons within the dorsal raphe nucleus (DRN) are necessary and sufficient to produce exaggerated fear and shuttle box escape deficits. In sedentary rats, IS-induced sensitization of these neurons leads to exaggerated 5-HT release in brain regions regulating anxiety during later behavioral testing. 6 weeks of exercise prior to IS can constrain the hyperactivation and sensitization of these neurons, thereby protecting against exaggerated 5-HT release and resulting behavioral deficits. Exercise in adulthood regulates 5-HT neuronal activity by increasing mRNA for 5-HT_{1a} inhibitory autoreceptors (5-HT_{1a}R) within the DRN, and decreases postsynaptic 5-HT_{2C} receptors (5-HT_{2C}R) within DRN projection regions important for anxiety and depressive-like behavior. The persistent stress protection produced by early life exercise could involve stable adaptations within 5-HT receptors and/or adaptive alterations within circuits involved in

regulating DRN 5-HT neuron activity. To test this, adult (PND 70) and juvenile (PND 24) male, Fisher (F344) rats were allowed access to a running wheel or remained sedentary for 6 weeks. Following cessation of exercise, rats were sacrificed and brains were collected either immediately or following 25 days to examine gene expression using *in situ* hybridization. Preliminary analyses revealed that early life exercise produces persistent increases in 5-HT_{1a}R mRNA expression within the DRN. Analysis of 5-HT_{2C}R receptors and plasticity markers in brain regions involved in regulating DRN 5-HT neuron activity is currently underway. These data suggest that early life exercise produces long lasting, adaptive alterations in stress responsive brain circuits, perhaps by capitalizing upon the pervasive plasticity of the developing brain.

Disclosures: A. Mika: None. **N.L. Rumian:** None. **A.K. Hills:** None. **S.O. McConnell:** None. **C.A. Bouchet:** None. **B.N. Greenwood:** None. **M.R. Fleshner:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.16/AA26

Topic: F.02. Animal Cognition and Behavior

Support: NIH Training Grant T32MH020068-14

Title: Early life stress alters the development of the fear circuit and fear learning in mice

Authors: *G. MANZANO-NIEVES[1], K. G. BATH[2];
[1]Dept. of Neuroscience, Brown Univ., Providence, RI; [2]Dept. of Cognitive, Linguistic and Psychological Sci., Brown Univ., Providence, RI

Abstract: Acute traumatic events and/or prolonged stress incurred early in life increase the risk of developing anxiety and depressive-like behaviors in both humans and animal models. In addition, recent work has suggested that early life stress can accelerate the development of the fear circuit in rats (Cowan CS et al., 2013; Moriceau S et al., 2009). However, the effect of early life stress (ELS) on neural development, and their consequences on circuit activation are not well known. To investigate the effect of ELS on the development of the conditioned fear circuit we took advantage of an ELS paradigm that consisted of reducing maternal access to bedding and nesting materials from postnatal days 4 to 11 (Rice CJ et al., 2008). Subsequently, we investigated the development of fear learning and expression in control reared and ELS mice using a standard cued fear conditioning paradigm. Briefly, mice were exposed to six tones, each of which co-terminated with a foot-shock (0.57mA) on postnatal day (P) 21, 28, or 50. Consistent with previous work, we observed no effect of age on tone fear memory recall in control mice (Den ML and Richardson R, 2013). However, at postnatal day 21 both ELS reared

male and female mice showed significantly diminished levels of freezing during recall testing at 6 hrs. (Freezing: 45% Controls vs. 17% ELS; $P < 0.01$) and 24 hrs. (Freezing: 65% Controls vs. 22% ELS; $P < 0.01$) post conditioning. We hypothesized that ELS alters the timing of development of key regions involved in fear consolidation and recall, impairing performance at P21. Ongoing experiments are aimed at understanding the neural structures involved in the observed ELS phenotype.

Disclosures: G. Manzano-Nieves: None. **K.G. Bath:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.17/AA27

Topic: F.02. Animal Cognition and Behavior

Support: National Basic Research Program of China (no. 2015CB856400)

National Basic Research Program of China (no. 2015CB553503)

Natural Science Foundation of China (no. 31230033)

Natural Science Foundation of China (no. 91432303)

Natural Science Foundation of China (no. 81221002)

Title: Predictable chronic mild stress during adolescence promotes fear memory erasure in adulthood

Authors: *J. DENG[1,2], C. CHEN[1,2], S. MENG[1,2], C. SUN[1,2], L. XU[1,2], Y. XUE[2], X. GAO[1,2], W. YAN[1,2], N. CHEN[2], J. SHI[2], L. LU[1,2,3];
[1]Sixth Hosp. of Peking Univ., Beijing, China; [2]Natl. Inst. on Drug Dependence, Peking Univ., Beijing, China; [3]Peking-Tsinghua Ctr. for Life Sci. and PKU-IDG/McGovern Inst. for Brain Research, Peking Univ., Beijing, China

Abstract: Objective: Numerous studies confirmed that early-life stress is a predisposing factor for mental illness, such as anxiety and posttraumatic stress disorder. However, our recent findings indicated that adolescent predictable chronic mild stress (PCMS) promotes resilience to depression- and anxiety-like behaviors in adulthood, indicating that PCMS in early life may improve the ability to cope with stressful experiences in adulthood. In the present study, we investigated whether PCMS during adolescence promotes the extinction-induced inhibition of conditioned fear responses in adulthood. Methods: During PCMS procedure, the rats were restrained for 5 min each day for 28 days (postnatal days 28-55). Contextual fear conditioning was used to investigate the effects of PCMS in adolescence on fear memory acquisition and

extinction. Protein levels of brain-derived neurotrophic factor (BDNF)-extracellular signal-regulated kinase 1/2 (ERK1/2) signaling and Bdnf DNA methylation were detected after PCMS. Intra- prefrontal cortex infusion of BDNF antibody or ERK1/2 inhibitor U0126 was conducted to investigate the relationship between BDNF-ERK1/2 signaling and the enhancement of fear extinction induced by PCMS exposure during adolescence. Results: We found that PCMS during adolescence facilitated the extinction and inhibited the reinstatement and spontaneous recovery of fear memory in adult rats, and this effect was still present 1 week after PCMS exposure. Moreover, PCMS in adolescence increased the activity of BDNF-ERK1/2 signaling in the infralimbic cortex (IL) but not prelimbic cortex in adulthood. Intra-IL infusion of BDNF antibody and the ERK1/2 inhibitor U0126 reversed the PCMS-induced enhancement of fear extinction. Lastly, we found that PCMS decreased DNA methylation of the Bdnf gene at exons IV and VI and elevated the mRNA levels of Bdnf in the IL. Conclusion: Our findings demonstrated that adolescent PCMS exposure promoted fear memory erasure in adulthood, and this effect may be achieved through methylation modification of the Bdnf gene in the medial prefrontal cortex.

Disclosures: **J. Deng:** None. **C. Chen:** None. **S. Meng:** None. **C. Sun:** None. **L. Xu:** None. **Y. Xue:** None. **X. Gao:** None. **W. Yan:** None. **N. Chen:** None. **J. Shi:** None. **L. Lu:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.18/AA28

Topic: E.05. Stress and the Brain

Support: RO1 NS28912

P50 MH096889

Title: Hyper-excitability and epilepsy generated by chronic early-life stress

Authors: ***A. SINGH**[1], C. M. DUBÉ[2], J. MOLET[2], A. IVY[2], P. M. MARAS[1], T. Z. BARAM[3]; [1]Pediatrics, [2]Anatomy/Neurobiology, [3]Pediatrics, Anatomy/Neurobiology, Neurol., Univ. of Calfornia-Irvine, Irvine, CA

Abstract: Rationale: Epilepsy is more prevalent in populations with high measures of stress, but the neurobiological mechanisms are unclear. Stress is a common precipitant of seizures in individuals with epilepsy, and may provoke seizures by several mechanisms including changes in neurotransmitter and hormone levels in the brain. Previous studies have shown that stress during sensitive periods early in life contributes to ‘brain programming’, influencing neuronal function and networks. However, it remains unclear if early-life stress influences limbic excitability and promotes epilepsy. Methods: Here, we employed an established, naturalistic

rodent model of chronic early-life stress (CES). The rat pups were stressed by the unpredictable and fragmented nurturing behavior of the dams resulting from the limited bedding and nesting material in the cages from postnatal day (P) 2-9 (controls were housed under standard conditions). From P11-15, animals were implanted with bipolar electrodes in both hippocampi, or bilaterally in amygdala, as well as with one electrode over the right fronto-parietal cortex. Chronic cortical and limbic video-EEGs were used to examine the contributions of stress to age-specific epilepsies and network hyper-excitability. To probe the underlying molecular mechanisms, we utilized *in situ* hybridization and qRT-PCR to measure mRNA levels of Corticotropin-releasing hormone (CRH). Immunocytochemistry was used to determine CRH peptide levels. Results: EEGs obtained from the control animals throughout development were normal and no seizures were observed. EEGs from the majority of rats experiencing CES demonstrated epileptic spikes and spike series, and 57% of CES rats developed seizures. Behavioral events resembling the human age-specific epilepsy infantile spasms occurred in 48% of the animals, accompanied by EEG spikes and/or electrodecrements. 9% of the animals developed limbic seizures that involved the amygdala. Probing for stress-dependent, endogenous convulsant molecules within amygdala, we examined the expression of the pro-convulsant neuropeptide CRH, and found a significant increase of amygdalar, but not cortical, CRH expression in adolescent CES rats. Conclusions: These data suggest that CES has long-lasting effects on brain excitability and may promote age-specific seizures and epilepsy. Whereas the mechanisms involved require further study, these findings provide important insights into environmental contributions to early-life seizures.

Disclosures: **A. Singh:** None. **C.M. Dubé:** None. **J. Molet:** None. **A. Ivy:** None. **P.M. Maras:** None. **T.Z. Baram:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.19/AA29

Topic: E.05. Stress and the Brain

Support: National Natural Science Foundation of China 81301152

National Natural Science Foundation of China 81171284

International Brain Research Organization

Research Fund for the Doctoral Program of Higher Education of China 20120001110046

Beijing Natural Science Foundation 7133259

Title: Stress during a critical postnatal period induces region-specific structural abnormalities and dysfunction of the prefrontal cortex via CRF1

Authors: X.-D. YANG[1], X.-M. LIAO[1], A. URIBE-MARIÑO[2], R. LIU[1], X.-M. XIE[1], Y.-A. SU[1], J.-T. LI[1], M. V. SCHMIDT[2], T.-M. SI[1], *X.-D. WANG[3];
[1]Inst. of Mental Health, Peking Univ., Beijing, China; [2]Max Planck Inst. of Psychiatry, Munich, Germany; [3]Inst. of Neuroscience, Zhejiang Univ., Zhejiang, China

Abstract: During the early postnatal period, environmental influences play a pivotal role in shaping the development of the neocortex, including the prefrontal cortex (PFC) that is crucial for working memory and goal-directed actions. Exposure to stressful experiences during this critical period may disrupt the development of PFC pyramidal neurons and impair the wiring and function of related neural circuits. However, the molecular mechanisms of the impact of early-life stress on PFC development and function are not well understood. In this study, we found that repeated stress exposure during the first postnatal week hampered dendritic branching and spine formation in layers II/III and V pyramidal neurons in the dorsal agranular cingulate cortex (ACd) of neonatal mice. In adulthood, postnatally stressed mice exhibited impaired structural plasticity in layer V, but not layer II/III, neurons in the ACd. Most importantly, concurrent blockade of corticotropin-releasing factor receptor 1 (CRF1) by systemic antalarmin administration during the stress exposure attenuated the region-specific effects of early-life stress and rescued stress-induced cognitive impairments. Moreover, the reduced length of apical dendrites of ACd layer V pyramidal neurons correlated with the degree of cognitive deficits in stressed mice. Our data highlight the importance of the time window of stress exposure on the development of subpopulations of PFC pyramidal neurons, and suggest that CRF1 modulates the adverse effects of early-life stress on structural plasticity and function of PFC.

Disclosures: X. Yang: None. **X. Liao:** None. **A. Uribe-Mariño:** None. **R. Liu:** None. **X. Xie:** None. **Y. Su:** None. **J. Li:** None. **M.V. Schmidt:** None. **T. Si:** None. **X. Wang:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.20/AA30

Topic: E.05. Stress and the Brain

Support: NIMH Silvio Conte Center 1P50MH094271

Title: Early life stress impairs Parvalbumin networks in mouse prefrontal cortex

Authors: *Z. YE[1], K. SCHAEFER[3], H. S. KNOBLOCH-BOLLMANN[2], T. K. HENSCH[2,4];
[1]Neurobio., [2]MCB, Harvard Univ., Cambridge, MA; [3]Harvard Col., Cambridge, MA;
[4]Neurobio., Boston Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Neuronal circuits controlling our perception and behaviors are radically shaped by early life experiences. In humans, early life stress (ELS) has been linked to negative behavioral consequences throughout life and compromised structure/function of the prefrontal cortex (PFC). However, the underlying cellular mechanisms of ELS have not been fully elucidated. Inhibitory parvalbumin (PV) expressing interneurons are particularly vulnerable to genetic or environmental insults associated with cognitive disorders. Whether and how PV networks in the medial PFC are affected by ELS, or if they mediate the behavioral consequences, remains unclear. Here, we first adapted a parental separation protocol in *Peromyscus Polionotus* (PO), a monogamous wild mouse species that provides bi-parental care to offspring. Female PO offspring subjected to ELS exhibited significantly increased anxiety-like behaviors in adulthood as compared to control (CTL) POs (n=9 for CTL, n=13 for ELS, $p<0.01$). Moreover, PV expression in the medial PFC was negatively correlated with their anxious behaviors (slope=-0.5814 ± 0.2436, p=0.027). No changes in anxiety behavior or PV expression were detected in male POs (n=7 for CTL, n=12 for ELS). To further examine a role for PV networks in the medial PFC following ELS, we characterized the developmental profile of PV+ interneurons in the C57Bl6/J laboratory mouse strain. Consistent with our findings in PO mice, after experiencing an ELS paradigm of fragmented care induced by limited nesting material, female (but not male) offspring exhibited increased anxiety (n=11 for CTL, n=6 for ELS, $p<0.05$) and impaired PV networks in the mPFC, as measured by both qPCR and immunohistochemistry. Notably, this sex-specific effect of ELS on anxiety behaviors may have been mediated by differential expression of BDNF in female and male mice after ELS. Taken together, our data reveal increased anxiety and impaired inhibitory prefrontal PV circuits in female animals across mouse species and stress paradigms.

Disclosures: Z. Ye: None. **K. Schaefer:** None. **H.S. Knobloch-Bollmann:** None. **T.K. Hensch:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.21/AA31

Topic: E.05. Stress and the Brain

Title: Chronic stress enhances the hyperthermia in response to acute restraint stress

Authors: *T. MIYAMOTO[1,2], Y. FUNAKAMI[1], E. KAWASHITA[1], A. NOMURA[1], N. SUGIMOTO[1], S. ICHIDA[1], A. KAWABATA[1];
[1]Kinki Univ. Sch. Pharm., Higashi-Osaka, Japan; [2]Dept. Pharm., Seichokai Fuchu Hosp., Izumi, Japan

Abstract: Stress responses are important biological reactions to maintain homeostasis particularly through the autonomic nervous system. Acute mental stress is known to raise body temperature through excitation of the sympathetic nervous system which regulates non-shivering thermogenesis in the brown adipose tissue (BAT). The mechanism of the acute stress-induced hyperthermia still remains unknown. Here, we investigated the effect of chronic stress on acute stress-induced hyperthermia, using the specific alternation of rhythm in temperature (SART)-stressed mice that show various chronic stress symptoms and are now considered a model for autonomic imbalance. Male ddY mice weighing 20-25 g were kept at room temperature (24 ± 1 °C) under a 12 h-light and dark cycle (lights on at 8:00 am) and allowed to access food and tap water freely. Mice were exposed to restraint for 1 hour as acute stress, and loaded with SART stress as chronic stress, as follows. The mice were transferred hourly between two cages maintained at 24°C and 4°C from 9:00 to 16:00 and housed in the 4°C cage from l6:00 to 09:00 overnight. These procedures were repeated for 8 days and stopped at 11:30 on the final day. Each mice was deeply anesthetized with isoflurane for blood collection, and serum corticosterone (CORT), noradrenaline (NA) and adrenaline (Ad) levels were measured. Rectal temperature was monitored during the 1-hour restraint stress. Serum NA and Ad, but not CORT, levels elevated in SART-stressed mice. Acute restraint increased serum CORT, but not NA or Ad, levels, a response being greater in SART-stressed mice than unstressed mice. SART stress did not change the body temperature, whereas the restraint stress-induced hyperthermia was greater in SART-stressed mice than unstressed mice. The selective β3 adrenoceptor antagonist, SR59230A, improved the increased hyperthermia in response to restraint stress in the SART-stressed mice, while diazepam, an anxiolytic drug, and indomethacin, a cyclooxygenase inhibitor, had no such inhibitory effect. These results suggest that chronic stress enhances the acute stress-induced responses including hyperthermia, and the underlying mechanism might involve the non-shivering thermogenesis in BAT via β3 adrenoceptor stimulation.

Disclosures: **T. Miyamoto:** None. **Y. Funakami:** None. **E. Kawashita:** None. **A. Nomura:** None. **N. Sugimoto:** None. **S. Ichida:** None. **A. Kawabata:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.22/AA32

Topic: E.05. Stress and the Brain

Support: TopFund R0001363

Title: Early life stress induces rapid and persistent alterations in limbic endocannabinoid system

Authors: ***P. ATSAK**[1,2], M. MORENA[3], C. A. OOMEN[1,2], M. N. HILL[3], B. ROOZENDAAL[1,2];

[1]Radboud Univ. Med. Ctr., Nijmegen, Netherlands; [2]Donders Inst. for Brain, Cognition and Behavior, Nijmegen, Netherlands; [3]Dept. of Cell Biol. and Anat., Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Early life stress (ELS), such as childhood neglect, creates life-long vulnerability to stress-related anxiety disorders. Recent evidence has underscored endocannabinoid dysregulations in anxiety disorders; nevertheless, the endocannabinoid system has received very little attention in the context of ELS. Here, we examined the immediate and delayed effects of ELS on the endocannabinoid system in limbic brain regions in male rats. We employed a limited-nesting-induced maternal neglect paradigm that took place between postnatal day 2 and 9 that resulted in a robust disturbance in maternal care and induced stress in pups as evidenced by higher plasma corticosterone levels. We determined the immediate effects of ELS in pups at postnatal day 9 and found significant reductions in 2-arachidonoylglycerol (2-AG) levels in the amygdala, hippocampus and prefrontal cortex (PFC) as well as in anandamide levels in the amygdala. Moreover, ELS decreased 2-AG levels in the hippocampus and amygdala while augmenting anandamide levels in the amygdala and PFC in adult animals. Further, to investigate the residual effects of ELS on endocannabinoid recruitment during stress, we exposed adult animals to an acute swim stress challenge for 15 minutes and subsequently determined endocannabinoid levels in limbic brain regions. We found that forced swim stress significantly reduces anandamide levels in the amygdala and PFC. Stress, as expected, induced a dramatic increase in 2-AG levels in the hippocampus; however, this was absent in rats with ELS history. To gain insight into the mechanism underlying the ELS-induced endocannabinoid alterations, we assessed the activity of major endocannabinoid catalyzing enzymes in the same brain regions. Moreover, to examine whether the changes in endocannabinoid ligand levels drive a permanent effect on the receptor type 1 (CB1), we evaluated CB1 sensitivity and expression in all these brain regions. We did not find any alterations in these parameters except elevated CB1 expression. These findings show that ELS induces endocannabinoid alterations in brain region- and time-dependent fashion under baseline conditions. Moreover, ELS history significantly disturbs the stress-induced recruitment of hippocampal endocannabinoid signaling in adults. These findings may carry important implications for understanding the mechanisms that mediate the life-long effects of ELS. Early 2-AG alterations could affect the cortical wiring of the brain whereas delayed alterations can lead to cognitive deficits or enhanced emotional memory that might predispose individuals to vulnerability to psychopathology such as post-traumatic stress disorder.

Disclosures: P. Atsak: None. **M. Morena:** None. **C.A. Oomen:** None. **M.N. Hill:** None. **B. Roozendaal:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.23/AA33

Topic: E.05. Stress and the Brain

Title: Early life stress accelerates behavioral and neural maturation of the hippocampus

Authors: *K. G. BATH[1], G. MANZANO-NIEVES[2], H. GOODWILL[2];
[1]CLPS, [2]Neurosci., Brown Univ., Providence, RI

Abstract: Early Life Stress (ELS) increases risk for cognitive and emotional dysfunction. ELS suppresses proliferation and increases cell death in postnatal hippocampus, leading to the prevailing hypothesis that ELS retards brain development. Recently, ELS has been associated with activating effects on behavioral and functional brain development, indicating potential consequences for the timing of brain development. However, the effect of ELS on measures of neural maturation have not been assessed. Here we used a mouse model of ELS, fragmented maternal care, and a cross-sectional dense sampling approach focusing on the hippocampus to directly measure the effects of ELS on the expression of genetic markers of neural maturation as well as the ontogeny of behavioral development. In a contextual fear-conditioning task, ELS accelerated the timed developmental suppression of contextual fear responding. Using RT-qPCR, we found that ELS led to a precocious switch in NMDA receptor subunit expression (marker of synaptic maturity), an earlier rise in parvalbumin expression (a late developing class of interneurons), and earlier expression of myelin basic protein (a key component of the myelin sheath). ELS led to a more rapid decline in expression of markers of cell proliferation and differentiation (Ki-67 and doublecortin). Together, these data are the first direct evidence to support the hypothesis that ELS serves to switch neurodevelopment from processes of growth to maturation. This represents a paradigm shift in our thinking regarding ELS effects on neurobehavioral development, and indicate that ELS may serve to promote earlier maturation of some circuits to cope with early adversity.

Disclosures: **K.G. Bath:** None. **G. Manzano-Nieves:** None. **H. Goodwill:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.24/AA34

Topic: E.05. Stress and the Brain

Support: CONACyT 221092

CONACyT 238313

Title: Hippocampal cytogenesis on stress-exposed senile rats: effects of previous early-life exposure to environmental stress

Authors: *T. P. MARTÍNEZ[1], D. RUESGA-BARCENAS[2], J. GARCÍA-ESTRADA[3], S. LUQUÍN[2], Y. RUVALCABA-DELGADILLO[2];
[1]Neurosci., Univ. De Guadalajara, Guadalajara, Mexico; [2]Neurosci., Univ. de Guadalajara, Guadalajara, Mexico; [3]Neurosci., Ctr. de Investigación Biomédica de Occidente, Guadalajara, Mexico

Abstract: The hippocampus is one of the few areas of the rodent brain that has the ability to continue producing new cells postnatally; also this area is closely related to stress effects. Changes in hippocampal proliferation have been discussed based on volumetric variations reported in chronically stressed subjects; however, it is unclear whether proliferative changes may affect senile subjects with a history of stress. In the present study, hippocampal cytogenesis in stress-exposed senile rats was assessed as a function of whether or not the rats were exposed to stress at early stages of life. The thymidine analog BrdU was administered to senile male wistar rats in order to evaluate DG, CA3, CA2 and CA1 proliferative changes. We found statistically significant elevations in the number of BrdU marked cells among the hippocampus of rats of stressed rats that were also exposed at early stages of life. Then, we provide evidence that early exposure to stress may change the way that older subjects respond to new stressing conditions.

Disclosures: T.P. Martínez: None. **D. Ruesga-Barcenas:** None. **J. García-Estrada:** None. **S. Luquín:** None. **Y. Ruvalcaba-Delgadillo:** None.

Poster

349. Developmental Regulators of Stressful Experiences

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 349.25/AA35

Topic: E.05. Stress and the Brain

Support: NSF Grant 1121886

NSF Grant 1258111

VA Merit Award 1I01BX001978

Georgia State University Brains and Behavior Program Seed Grant

Georgia State University Dissertation Grant

Chi Omega Foundation Alumnae Educational Grant

Title: Sex-dependent effects of early life inflammatory pain on energy homeostasis in adult rats

Authors: *M. B. PARENT[1,2], Y. O. HENDERSON[1], R. NALLOOR[3], A. VAZDARJANOVA[5,4], A. Z. MURPHY[1];
[1]Neurosci. Inst., [2]Dept. of Psychology, Georgia State Univ., Atlanta, GA; [3]Augusta Biomed. Res. Corp., [4]VA Res. Service, Charlie Norwood VA Med. Ctr., Augusta, GA; [5]Dept. of Pharmacol. and Toxicology, Georgia Regents Univ., Augusta, GA

Abstract: We hypothesize that dorsal hippocampal (dHC) neurons, which are critical for episodic memory, form a memory of a meal and inhibit meal onset during the postprandial period. In support, we showed previously that 1) consumption of a sucrose meal induces expression of the synaptic plasticity marker activity-regulated cytoskeleton-associated protein (*Arc*) in dHC neurons and 2) reversible inactivation of these neurons immediately following sucrose meal accelerates the onset of the next meal and increases the volume of intake. We have also previously reported that early life pain produces long-lasting dHC-dependent memory impairments in adult rats. The present study determined whether these memory deficits are accompanied by impaired energy homeostasis. Male and female Sprague-Dawley rats were given an intraplantar injection of the inflammatory agent carrageenan (1%) on the day of birth and their meal patterning and body mass were measured in adulthood. We found that neonatal inflammatory pain increased meal size in female and male rats, increased meal frequency in female rats, and prevented sucrose-induced *Arc* expression in dHC neurons in female rats, but not in male rats. Morphine administration at the time of injury attenuated the effects of injury. Injury also increased body mass at different ages in male and female rats; these sex- and age-dependent increases in body mass occurred concurrently with dHC-dependent memory deficits in the spatial water maze. Collectively, these findings indicate that one brief episode of neonatal inflammatory pain has a long long-lasting impact on energy homeostasis that is age- and sex-dependent and parallels dHC-dependent memory deficits.

Disclosures: M.B. Parent: None. **Y.O. Henderson:** None. **R. Nalloor:** None. **A. Vazdarjanova:** None. **A.Z. Murphy:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.01/AA36

Topic: E.07. Food Intake and Energy Balance

Title: Elucidating central mechanisms of the anorexigenic effect of neuropeptide K in broilers (Gallus gallus)

Authors: *J. YI, E. R. GILBERT, M. A. CLINE;
Virginia Tech., Blacksburg, VA

Abstract: The central mechanisms mediating anorexigenic effects of neuropeptide K (NPK) are poorly understood in chickens. Herein, we demonstrated the anorexigenic effect of NPK and central mechanisms associated with the hypothalamus. Intracerebroventricular (ICV) injection of NPK decreased food intake but had no effect on water intake in 4 day-old chicks. The c-Fos immunoreactivity was increased in the paraventricular nucleus (PVN) and arcuate nucleus in NPK-treated chicks, while no effect of treatment was observed in the lateral hypothalamus, ventromedial hypothalamus, dorsomedial hypothalamus, paraventricular nucleus, or the suprachiasmatic nucleus of the hypothalamus. Microinjection of NPK into the PVN of 14 day-old chicks also induced anorexia, demonstrating a primary site of action. Additionally, treatment with corticotropin releasing factor (CRF) receptor antagonists did not affect NPK-induced anorexia, which may indicate that CRF was not involved in NPK's anorexigenic effect. Gene expression of appetite-associated factors was measured in the hypothalamus after ICV injection and there was decreased agouti gene-related peptide (AgRP) mRNA abundance but no change in proopiomelanocortin, neuropeptide Y and oxytocin mRNA in NPK compared with vehicle-injected chicks. A comprehensive behavior analysis revealed a decreased number of feed pecks, exploratory pecks, jumps, escape attempts, and distance moved and increased time spent standing for chicks that were ICV injected with NPK. In conclusion, NPK is associated with appetite regulation in chickens and its anorexigenic effects may be mediated by the paraventricular nucleus and arcuate nucleus in the hypothalamus and involve AgRP.

Disclosures: J. Yi: None. **E.R. Gilbert:** None. **M.A. Cline:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.02/AA37

Topic: E.07. Food Intake and Energy Balance

Title: Elucidating the anorexigenic mechanism of central oxytocin in birds

Authors: *B. MCCONN, E. R. GILBERT, P. B. SIEGEL, M. A. CLINE;
Virginia Tech., Blacksburg, VA

Abstract: Oxytocin (OT) is a well characterized neurotransmitter that participates in a wide range of physiological effects such as inhibition of food intake, although the mechanism mediating this response is poorly understood in any species, especially in non-mammalian vertebrates. Thus, this study was designed to determine the effects of central OT injection on food intake in 4 day-old 3-h-fasted chicks (Gallus gallus). Intracerebroventricular (ICV) injection of 0.625, 2.5, 5.0, and 10.0 nmol OT decreased food and water intake during the 180 min observation period. Drink was not prandial as chicks that were food-restricted after ICV OT still reduced their water intake. There was increased c-Fos immunoreactivity in several appetite-

associated hypothalamic nuclei after ICV OT, including the arcuate (ARC), dorsomedial nucleus (DMN), lateral hypothalamus (LH), paraventricular nucleus (PVN), and ventromedial hypothalamus (VMH). We then investigated appetite-associated effects of OT in chicks from lines that have been genetically selected for either low (anorexic-containing) or high (all obese) body weight. Central OT decreased food intake in both lines with the magnitude of response greater in the high than low weight line. In conclusion, ICV OT induced anorexia, reduced water intake, and was associated with activation of the ARC, DMN, LH, PVN, and VMH in the hypothalamus. Chicks from the line selected for high body weight were more responsive to the anorexigenic effects of OT.

Disclosures: **B. McConn:** None. **E.R. Gilbert:** None. **P.B. Siegel:** None. **M.A. Cline:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.03/AA38

Topic: E.07. Food Intake and Energy Balance

Support: NIDA Grant 02650-70699

Title: Expression patterns of BDNF with central anorexigenic signaling pathways involving PACAP in the hypothalamic ventromedial nuclei

Authors: *B. MAUNZE, M. HURLEY, J. M. RESCH, M. J. REILLEY, E. M. WASSMANN, S. CHOI;
Marquette Univ., Milwaukee, WI

Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 38-amino acid polypeptide belonging to the secretin super family of peptides. PACAP binds to its type 1 receptor (PAC1R) with greater affinity than for the receptors for vasoactive intestinal polypeptides (VIP), VPAC1 and VPAC2. Although mRNA for PACAP and its receptor PAC1R are found throughout the central nervous system, they are abundantly expressed in the hypothalamic ventromedial nuclei (VMN). In male Sprague Dawley rats, infusions of PACAP into the VMN produce a robust decrease in food intake with concomitant increased energy expenditure, decreased body weight, and significantly elevated brain-derived neurotrophic factor (BDNF) mRNA expression in the VMN. This latter effect of PACAP on BDNF mRNA expression has been shown to occur in other brain regions. Exogenous BDNF in the VMN regulates energy homeostasis in a manner similar to that of PACAP with decreased feeding and increased metabolism. Although the physiological responses to individual PACAP and BDNF infusions in the VMN lead to decreased feeding behavior and body weight loss, the anatomical distribution of these two cell signals in the VMN has not been established. PACAP-induced

changes in BDNF mRNA expression in the VMN may reveal an important interaction with PACAP signaling in the control of feeding behavior. In the present study, we have employed double-labeled fluorescent in-situ hybridization (FISH) to examine the expression patterns of PACAP, PAC1R and BDNF mRNA containing neuronal cells. In the VMN, PACAP mRNA expressing cells co-express BDNF, PAC1R, and VGLUT2. BDNF mRNA expressing cells co-express PAC1R and PACAP. Coupled with previous behavioral data demonstrating PACAP- and BDNF-induced changes in feeding behavior, the co-expression of BDNF with PACAP and PAC1R mRNA in the VMN suggest a potential functional relationship between the two signaling peptides in the regulation of energy homeostasis. The specific and integrated contributions of PACAP and BDNF in the VMN towards regulating energy homeostasis and feeding behavior still remain to be studied.

Disclosures: B. Maunze: None. **M. Hurley:** None. **J.M. Resch:** None. **M.J. Reilley:** None. **E.M. Wassmann:** None. **S. Choi:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.04/AA39

Topic: E.07. Food Intake and Energy Balance

Title: Peripheral administration of glucagon-like peptide-1 and cholecystokinin-8 activates nesfatin-1-containing neurons in the hypothalamus and brainstem of rats

Authors: *Y. YAMAMOTO[1], R. SAITO[1], M. SO[2], Y. MOTOJIMA[2], T. MATSUURA[2], M. YOSHIMURA[2], H. HASHIMOTO[2], K. KUSUHARA[1], Y. UETA[2];
[1]Univ. Occupat & Environ Hlth., Kitakyushu city, Japan; [2]Dept. of Physiol., Univ. of Occup. and Envrn. Hlth., Kitakyushyu, Japan

Abstract: Peripheral anorectic hormones,glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK)-8 and leptin suppress food intake. Newly identified anorectic neuropeptide, nesfatin-1 is synthesized not only in the peripheral tissue but also in the central nervous system, in particular various nuclei in the hypothalamus and brainstem. In the present study, we examined the effects of intraperitoneal (ip) administration of GLP-1, CCK-8 and serial ip administrations of GLP-1 and leptin at subthreshold dose against feeding in each on nesfatin-1-immunoreactive (ir) neurons in the hypothalamus and brainstem of rats, using immunohistochemistry for Fos. Ip administration of GLP-1 (100 μg/kg) caused significant increases of nesfatin-1-ir neurons expressing Fos-ir in the supraoptic nucleus (SON), the area postrema (AP) and the nucleus tractus solitarii (NTS) but not in the paraventricular nucleus (PVN), the arcuate nucleus (ARC) and the lateral hypothalamic area (LHA). On the other hand, marked increases of nesfatin-1-ir neurons expressing Fos-ir were observed in the SON, PVN, AP and NTS but not the ARC and

LHA after ip administration of CCK-8 (50 μg/kg). There were no differences in percentage of nesfatin-1-ir neurons expressing Fos-ir in above nuclei of the hypothalamus and brainstem among saline-, GLP-1 (33 μg/kg)- and leptin (300 μg/kg)-treated rats. However, serial administrations 37 of GLP-1 (33 μg/kg) and leptin (300 μg/kg) caused significant increases of nesfatin-1-ir neurons expressing Fos-ir in the AP and NTS. These results suggest that nesfatin-1-containing neurons in the brainstem may have an important role to sense peripheral levels of GLP-1 and leptin as well as CCK-8.

Disclosures: Y. Yamamoto: None. **R. Saito:** None. **M. So:** None. **Y. Motojima:** None. **T. Matsuura:** None. **M. Yoshimura:** None. **H. Hashimoto:** None. **K. Kusuhara:** None. **Y. Ueta:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.05/AA40

Topic: E.07. Food Intake and Energy Balance

Title: Deletion of melanin-concentrating hormone receptor 1 from GABAergic neurons increases locomotor activity

Authors: *M. J. CHEE, S. E. FLAHERTY, III, N. BRIANCON, J. S. FLIER, E. MARATOS-FLIER;
Endocrinol., BIDMC, Harvard Med. Sch., Boston, MA

Abstract: Melanin-concentrating hormone (MCH) is an orexigenic neuropeptide produced exclusively in the lateral hypothalamus. It is a critical regulator of energy balance and acutely stimulates food intake, leading to increased body weight gain. Transgenic mouse models targeting the MCH system revealed that the loss of MCH or its receptor MCHR1 increased energy expenditure and locomotor activity. MCHR1 expression is widespread but the function and contribution of first-order MCH-responsive neurons is not known. We recently showed that the highest density of MCHR1-expressing cells is GABAergic, especially in the accumbens nucleus. Furthermore, the accumbens partly mediates MCH locomotor actions. By selectively deleting MCHR1 from cells expressing the vesicular GABA transporter (vGAT), we tested if GABAergic neurons mediate MCH effects on body weight, energy expenditure or locomotor activity. We first generated the *MCHR1-flox* mouse and crossed it to the *vGAT-cre* mouse; the resulting conditional knockout is designated as *vGAT-MCHR1-KO*. *In situ* hybridization showed the absence of MCHR1 from GABAergic regions, most notably in the caudate putamen, accumbens and arcuate nucleus. MCHR1 hybridization was unaffected in glutamatergic regions, including the pyramidal cells in the cerebral cortex and hippocampus and paraventricular

hypothalamic nucleus. We next compared differences in body weight and locomotor activity of female *vGAT-MCHR1-KO* mice to their *vGAT-cre* controls. *vGAT-MCHR1-KO* mice were 11% leaner, with 20% less body fat, and had 70% greater total energy expenditure. However the most impressive difference was their 93% increase in total baseline ambulation. Blocking dopamine reuptake with GBR12909 systemically induces hyperactivity. Consistent with our previous report that increased dopaminergic tone underlies the hyperactivity of MCH-deficient mice, we found that *vGAT-MCHR1-KO* mice had an enhanced and prolonged response to GBR12909, which produced a two-fold increase in cumulative activity lasting more than 5 hours. In aggregate, these results indicate that MCH acts partly via inhibitory GABAergic neurons to regulate body weight and energy expenditure. Furthermore, these findings indicate that MCHR1 activation on GABAergic neurons regulate dopaminergic tone that underlie MCH-mediated ambulatory activity. The identity of the specific GABAergic neurons underlying MCH-mediated ambulatory activity is not known. However we may infer that those critical GABAergic cells will receive strong dopaminergic projections. We speculate that MCH-mediated GABAergic signaling from accumbens neurons inhibits mesolimbic dopamine transmission.

Disclosures: **M.J. Chee:** None. **S.E. Flaherty:** None. **N. Briancon:** None. **J.S. Flier:** None. **E. Maratos-Flier:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.06/AA41

Topic: E.07. Food Intake and Energy Balance

Support: NIH grant NS041669,

NIH grant AG019206

NIH grant NS045016

Title: MANF is a neurotrophic factor involved in the regulation of feeding behavior and energy homeostasis

Authors: ***S. YANG**, S. LI, X.-J. LI;
Human Genet., Emory Univ., Atlanta, GA

Abstract: MANF is a newly identified, ubiquitously expressed neutrophic factor that is found to be neuro-protective in several disease models, including Parkinson's Disease, stroke and Spinocerebellar Ataxia 17. Emerging evidence suggests that MANF is an ER stress inducible protein, and possesses anti-apoptotic properties. However, the exact cellular functions of MANF, especially in the central nervous system, remain poorly understood. We have generated a

transgenic mouse model, which overexpresses MANF in the central nervous system via mouse prion promoter. Western blotting and immunohistochemistry confirmed that transgenic MANF is expressed in various brain regions. Interestingly, MANF transgenic mice developed significant obesity at the age of 4-month. By examining food intake, we found that the increased adiposity in transgenic MANF mice is due to hyperphagia, suggesting that MANF may be important for regulating feeding behavior. We are currently in the process of studying molecular mechanisms underlying MANF-mediated feeding behavior.

Disclosures: **S. Yang:** None. **S. Li:** None. **X. Li:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.07/AA42

Topic: E.07. Food Intake and Energy Balance

Support: VA Grant to D.M.K. No. RX000458

Title: Energy homeostasis and food intake alterations in mice lacking central serotonin

Authors: ***M. ANGOA-PEREZ**[1,2], J. G. GRANNEMAN[2,3], R. G. MACKENZIE[1], D. M. KUHN[1,2];
[1]Dept. of Psychiatry and Behavioral Neuroscien, Detroit, MI; [2]Res., Res. & Develop. Service (11R), John D. Dingell VA Med. Ctr., Detroit, MI; [3]Pathology, Wayne State Univ., Detroit, MI

Abstract: Energy homeostasis and feeding regulation are important adaptive responses critical for survival. Neuropeptide Y (NPY) is an important central regulator of food consumption and energy expenditure. This 36-aminoacid peptide is highly expressed in the hypothalamus but also can be found in other areas including the hippocampus and thalamus. Mammalian studies have shown a link between serotonin and NPY in the acute regulation of feeding and energy balance and central serotonin is considered a powerful anorexic agent. Therefore, we aimed to investigate the effects of the lack of brain serotonin on NPY expression and food intake. For this, we used mice genetically depleted of tryptophan hydroxylase-2 (TPH-2), the rate limiting enzyme in the synthesis of brain serotonin. We assessed body weight and food intake under ad libitum conditions and after periods of fasting in TPH-2 knockout (KO) and wild type (WT) control mice. Expression of NPY in the hypothalamus, hippocampus and thalamus was also evaluated in both genotypes. The results showed a significant decrease in expression of NPY in hippocampus, thalamus and hypothalamus in KO animals compared to WT controls. KO mice showed an increase in food intake under normal conditions but body weight was not significantly increased in these mice. KO mice showed a greater drop in body weight after fasting compared to WT mice. In addition, food consumption of KO mice was higher after being allowed to eat for 4

hours after fasting but normalized by 24 h. These results indicate that central serotonin is involved in the expression of NPY and consequently in the modulation of food intake and energy homeostasis.

Disclosures: **M. Angoa-Perez:** A. Employment/Salary (full or part-time):; Department of Psychiatry, School of Medicine, Wayne State University. **J.G. Granneman:** A. Employment/Salary (full or part-time):; Department of Pathology, Wayne State University. **R.G. MacKenzie:** A. Employment/Salary (full or part-time):; Department of Psychiatry, School of Medicine, Wayne State University. **D.M. Kuhn:** A. Employment/Salary (full or part-time):; Department of Psychiatry, School of Medicine, Wayne State University.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.08/AA43

Topic: E.07. Food Intake and Energy Balance

Support: UNAM-DGAPA PAPIIT IN224214

FESI PAPCA-2014-54

Title: Changes in the immunoreactivity of c-Fos and α-MSH in the arcuate nucleus by activation of MC3 receptors

Authors: *D. DIAZ-URBINA, F. CORTES-SALAZAR, J. O. SUAREZ-ORTIZ, R. E. ESCARTÍN-PEREZ, V. E. LOPÉZ-ALONSO, J. M. MANCILLA-DÍAZ;
Facultad De Estudios Superiores Iztacala- UNAM, Tlalnepantla, Mexico

Abstract: The aim of the present work was evaluate the effects of the activation of cerebral MC3 melanocortin receptors (MC3r) on food intake (high-fat, HF) and the behavioral satiety sequence (BSS), as well as on c-Fos and α-MSH immunoreactivity in the arcuate nucleus of the hypothalamus (ARC). METHODS: Forty male Wistar rats (200-230g) were feed with a HF diet (45% kcal from fat), and after 2 days of habituation, animals were esteriotaxically implanted with guide cannulas in the lateral ventricle. After 3 days of post-surgery recovery, experimental subjects had access to the HF diet during 10 additional days. At the end of the HF diet exposure (10th day, 1h before the onset of the dark cycle) rats received intra-ventricular injections of vehicle (saline+saline, control group), MC3r agonist (saline+[D-trp8]-γ-MSH, agonist group), or MC3r antagonist (SHU9119+saline, antagonist group). After i.c.v injections, rats were video-recorded during 1 h and the analysis of BSS was conducted. Finally, rats were sacrificed and their brains extracted and processed for immunohistochemistry. RESULTS: Intra-ventricular administration of [D-trp8]-γ-MSH decreased food intake (g) in comparison to control group. In contrast, administration of SHU9119 stimulated HF diet consumption (g) compared to the

agonist group. Moreover, injection of [D-trp8]-γ-MSH reduced the number of α-MSH-positive neurons in the ARC, while intra-ventricular injection of SHU9119 increased the number of c-Fos-positive neurons and decreased α-MSH- immunoreactivity in the ARC. According to the BSS analysis, i.c.v administration of [D-trp8]-γ-MSH induced disruption of the satiety process (BSS). On the contrary, injection of SHU9119 did not altered typical behavioral pattern of BSS. CONCLUSION. Activation of MC3r reduced intake of a HF diet by interrupting the satiety sequence, and this effect may be related to the reduction of α-MSH immunoreactivity in the ARC. On the other hand, antagonism of MC3r stimulated HF diet consumption and preserved the behavioral pattern of the BSS, probably as a result of the reduction of the α-MSH and/or by activation of orexigenic neurons in the ARC (as revealed increase of c-Fos immunoreactivity).

Disclosures: D. Diaz-Urbina: None. **F. Cortes-Salazar:** None. **J.O. Suarez-ortiz:** None. **R.E. Escartín-Perez:** None. **V.E. Lopéz-Alonso:** None. **J.M. Mancilla-Díaz:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.09/AA44

Topic: E.07. Food Intake and Energy Balance

Support: CIHR (Canadian Institutes of Health Research)

OMHF (Ontario Mental Health Foundation)

Title: Ghrelin receptors in the VTA mediate stress induced changes in caloric intake during chronic social defeat

Authors: *S.-B. PARK[1], T. RODRIGUES[1], C. WALLACE[2], K. MEZHER[1], L. HYLAND[1], M. KLEIN[3], A. EDWARDS[1], Z. R. PATTERSON[1], H. MACKAY[1], A. ABIZAID[1];
[1]Neurosci., [2]Psychology, Carleton Univ., Ottawa, ON, Canada; [3]Pharmacol., Biomed. Sci. Institute, Univ. of São Paulo, São Paulo, Brazil

Abstract: Ghrelin, a gut-derived peptide hormone, is associated with feeding, energy balance, and the stress response. Exposure to stressors lead to elevated plasma levels of ghrelin, and these are associated with metabolic changes generated to meet the demands of the stressor. In mice, exposure to chronic social defeat leads to increases in caloric intake, and in particular, the intake of carbohydrate rich diets, and these effects are mediated in part by increased ghrelin secretion. It is not, however, the site at which ghrelin acts to mediate this effect. To determine this, we examined differences in the expression of the ghrelin receptor (GHSR) in regions of the brain that are associated with food intake, and the stress response including the ventral portion of the hypothalamus (vHYP), ventral tegmental area (VTA), prefrontal cortex (PFC), and the hippocampus (HIPP). As expected, exposure to chronic social defeat resulted in elevated plasma

ghrelin level ($p. < 0.05$), and significant changes in GHSR mRNA expression that were site dependent. Of these, the VTA was the only region where GHSR mRNA expression was significantly elevated ($p. < 0.05$). To examine the relevance of elevated GHSR expression in the VTA, we conducted an experiment where animals received a GHSR antagonist (JMV2959) or vehicle chronically infused into the VTA via cannula attached to the osmotic minipump while being stressed. Stressed mice treated with JMV2959 showed attenuated intake of the carbohydrate rich diet compared to stressed animals receiving vehicle, suggesting that GHSR in the VTA is important for the increase in carbohydrate intake during chronic social defeat.

Disclosures: S. Park: None. **T. Rodrigues:** None. **C. Wallace:** None. **K. Mezher:** None. **L. Hyland:** None. **M. Klein:** None. **A. Edwards:** None. **Z.R. Patterson:** None. **H. MacKay:** None. **A. Abizaid:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.10/AA45

Topic: E.07. Food Intake and Energy Balance

Support: WesternU Research Funds

Title: Emesis elicited by thapsigargin administration in the least shrew is due to activation of ERK1/2 signaling and Substance P release in the brainstem

Authors: W. ZHONG, S. CHEBOLU, *N. A. DARMANI;
Coll Osteo. Med. Pacific, Western Univ. Hlth. Sci., Pomona, CA

Abstract: Thapsigargin inhibits the Ca2+-transporter ATPase mediated uptake of Ca2+ into sarcoplasmic/endo-plasmic reticulum, thereby enhancing the cytoplasmic free concentration of Ca2+. Previously we had proposed that Ca2+ mobilization functions as a critical factor in the induction of emesis. In the present study, we established that i.p. injection of thapsigargin induces vomiting in a bell-shaped manner in the least shrew, with maximal efficacy (100%) at its 0.5 mg/kg dose. We then explored the potential mechanisms associated with this observation. As shown by immunohistochemistry, thapsigargin (0.5 mg/kg) triggered the activation of c-Fos and pERK expression in the brainstem dorsal vagal complex (DVC) emetic nuclei including the area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMNX). In addition, thapsigargin (0.5 mg/kg, i.p.) led to enhancements of 5-HT and Substance P (SP) immunoreactivity in brainstem sections. We then assessed the antiemetic potential of a selective 5-HT3- and NK1 -receptor antagonist, palonosetron and netupitant respectively. Nutepitant but not palonosetron attenuated evoked emetic parameters, suggesting that thapsigargin-elicited vomiting is mediated by NK1Rs and is independent of 5-HT3R activation. In addition,

pretreatment with the ERK inhibitor PD98059 (5 mg/kg., i.p.) 30 min prior to thapsigargin administration abolished thapsigargin-evoked responses including increases in SP content and vomiting behavior. In sum, this is the first study to demonstrate that the Ca2+ mobilizing agent thapsigargin, induces emesis, via the ERK-SP-NK1R pathway in the brainstem. This work was supported by WesternU funds.

Disclosures: **W. Zhong:** None. **S. Chebolu:** None. **N.A. Darmani:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.11/AA46

Topic: E.07. Food Intake and Energy Balance

Support: FONDECYT REGULAR 1150274

PROYECTO ATRACCION CIENTIFICOS DESDE EL EXTRANJERO CONICYT 82130017

PROYECTO INVESTIGACION REGULAR UNAB DI-523-14/R

Title: Impact of cafeteria diet and voluntary exercise on behavioural effects of dynorphin and orexin peptides in the PVN

Authors: *C. E. PEREZ-LEIGHTON, L. GAC;
Univ. Andres Bello, Santiago, Chile

Abstract: The orexin/dynorphin (ox/dyn) neurons release both orexin and dynorphin, neuropeptides that affect food intake and energy expenditure. While the orexins peptides have been extensively studied, less is known about the role of dynorphin peptides released from these neurons. How orexin and dynorphin peptides act together to modulate energy balance is unclear. The hypothalamic paraventricular nucleus (PVN) is important to feeding behavior and physical activity, and recent work from our laboratory has focused on the interaction between orexin and opioid/non-opioid dynorphin peptides in the PVN. To understand how obesity modulates the effects of orexin and dynorphin peptides on food intake, we cannulated Balb/c mice (n = 16) aiming at the PVN. Next, using a repeated measured design, orexin-A (0, 200, 400 pmol) and the opioid DYN-A_{1-13} (0, 0.625, 1.25, 2.5, 5 nmol) were injected into PVN before and after feeding with either a control diet (standard rodent chow, n = 8) or cafeteria diet (CAF diet, n = 8) for 10 weeks. Prior to the dietary interventions, DYN-A_{1-13} and orexin-A both significantly increased short-term chow intake (2h post-injection, one-way repeated measured ANOVA, $P < 0.05$). Mice fed CAF diet were classified as either obesity prone (OP, n = 4) or obesity resistant (OR, n = 3) based on whether their increase in fat mass percent was higher (OP) or lower (OR) compared to

maximum value of control fed mice. In OP and control diet-fed mice, we observed no change in the ability of orexin-A or DYN-A_{1-13} to increase short-term chow intake relative to the pre-dietary treatment (paired t-test per dose, $P > 0.05$). However, in OR mice we observed a trend toward increased ability of orexin-A to promote short-term chow intake (paired t-test, orexin-A at 400 pmol dose, $P = 0.13$) and no trends for changes in the effectiveness of DYN-A_{1-13}. Currently we are repeating this experiment and extending these results by testing if voluntary physical activity (wheel running) alters gene expression of the orexin and dynorphin genes in the ox/dyn neurons in the lateral hypothalamus, and whether these changes influence the behavioral effects of orexin and dynorphin peptides injected into PVN. These experiments will improve our understanding of the mechanisms by which the orexin/dynorphin neurons control energy balance.

Disclosures: C.E. Perez-Leighton: None. **L. Gac:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.12/AA47

Topic: E.07. Food Intake and Energy Balance

Support: DA 035088

Title: A new obesity model reveals the hypophagic properties of PACAP involve the regulation of homeostatic feeding in the ventromedial hypothalamic nucleus and hedonic feeding in the nucleus accumbens

Authors: *M. M. HURLEY, B. MAUNZE, J. M. RESCH, M. M. FRENKEL, M. J. REILLY, M. BLOCK, D. A. BAKER, S. CHOI;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Binge eating in humans is a complex disorder that often involves discrete, compulsive feeding sessions of highly palatable foods even in the absence of a deprivation state or hunger. Binging can be effectively modeled in rodents by providing subjects with limited access to a palatable food source (Western Diet; WD) as an adjunct to ad lib access to normal chow (Standard Chow; SC). Although this design recapitulates several fundamental characteristics observed in binge eating disorder, the binge eating observed in this paradigm is likely a product of both hedonic and homeostatic drives with the need to balance energy stores still present. To isolate these feeding drives, we have developed a novel feeding regimen that modifies the classic limited access binge model to effectively delineate and separate homeostatic feeding from motivational feeding. This is achieved by entraining male Sprague-Dawley rats to a restricted feeding schedule (two hours per day) of SC followed by a short 15 minute limited access meal of

either SC or WD (Restrict Fed-Limited Access; RFLA). The RFLA paradigm allows for the examination of pituitary adenylate-cyclase activating polypeptide (PACAP) on palatable food consumption in a fully satiated subject. PACAP has previously been shown to suppress feeding behavior when injected into the hypothalamus. In the current study, PACAP injected into the ventromedial hypothalamic nuclei (VMN) suppressed the two hour homeostatic SC meal, but not the subsequent 15 minute limited access meal of WD. By contrast, PACAP bilaterally administered into the nucleus accumbens (NAc) produced the opposite effect with PACAP suppressing the consumption of WD but not SC. Thus, PACAP mediated signaling in the VMN appears to be involved in homeostatic regulation of energy stores, whereas PACAP signaling in the NAc regulates feeding driven by palatability or hedonic qualities.

Disclosures: **M.M. Hurley:** None. **B. Maunze:** None. **J.M. Resch:** None. **M.M. Frenkel:** None. **M.J. Reilly:** None. **M. Block:** None. **D.A. Baker:** None. **S. Choi:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.13/AA48

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DDK83449

NIH Grant DDK41301

Title: Synergistic interaction between cholecystokinin agonist, (pGlu-Gln)-CCK-8 and urocotin-1 injected intraperitoneally on feeding in rats

Authors: *L. WANG, Y. TACHÉ, J. R. REEVE, Jr.;
UCLA, Los Angeles, CA

Abstract: Cholecystokinin (CCK) is one of the gut hormones signaling the brain to induce short term satiety (Dockray GJ, J. Physiol. 2014), and urocortin (Ucn)-1, a neuropeptide of corticotropin-releasing factor family reported to inhibit food intake when administered systemically in rodents (Wang L et al., AJP 2001, Peptides 2011; Hope PJ et al., 2000). Previously we showed that Ucn-1 or Ucn 2 has synergistic effect with CCK to inhibit food intake in overnight-fasted mice (Gourcerol G et al., 2007). (pGlu-Gln)-CCK-8 sulfated is a long acting CCK agonist shown in mice to reduce food intake and body weight (Irwin N et al., Diabetology 2012, Biochim Biophys Acta 2013). In this study, we assessed the synergistic effect of (pGlu-Gln)-CCK-8 and Ucn-1 on food intake and meal pattern of nocturnal feeding in non-fasted rats using an automated episode feeding monitoring system. SD rats were acclimated for 1 week to the feeding monitoring system (BioDAQ, Research Diets, Inc.). Peptides and vehicle (saline) were injected intraperitoneally (ip) before the onset of the dark phase. (pGlu-Gln)-CCK-8 was

tested first for effective doses at 0.3, 1 and 2.5 μg/kg (0.2, 0.6 and 1.8 nmol/kg). (pGlu-Gln)-CCK-8 at 0.6 and 2.5 μg/kg reduced the 2 h dark phase food intake by 48% and 78%, respectively compared to ip saline. Meal structure analysis showed significantly reduced meal size (-36% and -77%) and frequency (-41% and -53%), bouts (-46% and -68%) and time spent on meals (-53% and -69%) and prolonged latency to the 1st meal (69.9 ± 18.4 and 96.0 ± 13.3 vs 5.4 ± 2.8 min in ip saline). The changes in meal structures were no longer observed in 4-h cumulative feeding. Neither (pGlu-Gln)-CCK-8 at 0.3 μg/kg nor Ucn-1 at 10 μg/kg showed significant effect on the 2 h food intake and meal pattern. The peptides co-injection reduced the 2-h food intake by 60%, meal size by 66% and rate by 45%, and prolonged the onset of the first meal (60.5 ± 18.3 vs. 0.5 ± 0.1 min). Ucn-1 alone had a delayed inhibitory effect on the 4-h cumulative food intake (-32%) and meal size (-29%) and rate (-20%), and (pGlu-Gln)-CCK-8 plus Ucn-1 resulted in a higher inhibitory response (-48%, -46% and -37% respectively) which did not reach significance compared to Ucn-1. During the 12 h of dark phase, there was a significant synergistic reduction of the cumulative food intake (-20%) and average meal size (-36%). However, the satiety ratio was not significantly changed. These data indicate that Ucn-1 and (pGlu-Gln)-CCK-8 potentiate each other to induce a long-lasting reduction of dark phase food intake with alterations of meal structures (delayed onset of feeding and reduced meal size and eating rate) in rats, indicative of inhibition of orexigenic signals.

Disclosures: L. Wang: None. **Y. Taché:** None. **J.R. Reeve:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.14/BB1

Topic: E.07. Food Intake and Energy Balance

Support: NIH R01MH104261

ONR N00014-12-1-0366

Hope for Depression Research Foundation

Pritzker Neuropsychiatric Research Consortium

Title: The opioid beta-endorphin in the arcuate nucleus is involved in food intake

Authors: *Q. WEI, S. MOORE, S. WATSON, G. MURPHY, H. AKIL;
Mol. Behav. Neurosci. Inst., Ann Arbor, MI

Abstract: The arcuate nucleus (Arc) of the hypothalamus is an important site for the central regulation of food intake, energy expenditure, and body weight. There are two classes of peptide-producing neuronal populations in the arcuate nucleus that are thought to exert opposing actions

on feeding -- the orexigenic neurons that express NPY and AGRP, and the anorexigenic neurons that express POMC and CART. However, it should be noted that the gene encoding the precursor proopiomelanocortin (POMC) gives rise to two different groups of peptides, the melanocortins and beta-endorphin that are putatively co-released at axon terminals, and yet are thought to have opposing effects on feeding behavior. The role of melanocortins in the regulation of feeding and metabolism has been well defined by pharmacological and genetic methods, indicating an anorectic effect. On the other hand, pharmacological studies have generally indicated that opioids stimulate food intake, the opposite effect of melanocortins. While these components have been studied in isolation, we are unaware of studies that have selectively activated POMC neurons directly, thereby causing the release of both melanocortins and beta-endorphin while studying the impact on food intake. Thus, the current study used optogenetic stimulation to examine the effects of activating POMC neurons in the Arc on food intake in mice. In addition, we studied the impact on pain regulation, since this is one of the classical and well established effects of beta-endorphin on behavior. Surprisingly, activation of both dorsal and lateral Arc or of the lateral Arc alone led to a rapid and dramatic increase in food intake. This increased feeding could be largely blocked by pretreatment with the opioid receptor antagonist, naloxone, in a dose-dependent manner. Activation of both the dorsal and lateral Arc also induced an acute analgesic effect, which could be completely blocked by naloxone. In contrast, there was no analgesic effect of optogenetic stimulation in the lateral Arc. We also evaluated the cFos response following optogenetic stimulation in the Arc. Activation of the Arc resulted in an intensive cFos response in several brain regions. Importantly, the cFos response in these brain areas was abolished by pretreatment with naloxone. Together our findings demonstrate that the acute increase in food intake following activation of the arcuate is mediated by activation of the opioid system, and likely engages an extensive neural system in the brain. The exact physiological conditions that engage the appetite enhancing and appetite suppressive effects of POMC neurons remain to be fully understood.

Disclosures: **Q. Wei:** None. **S. Moore:** None. **S. Watson:** None. **G. Murphy:** None. **H. Akil:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.15/BB2

Topic: E.07. Food Intake and Energy Balance

Support: R00-DK090101

R01-DK103808

NIH Grant 5P60-DK020572

Title: Neurotensin receptor 1-expressing neurons in the ventral tegmental area modify energy balance

Authors: *H. M. BATCHELOR[1], H. WOODWORTH[1], J. BROWN[1], R. BUGESCU[1], P. FULLER[2], G. LEINNINGER[1];
[1]Michigan State Univ., East Lansing, MI; [2]Neurol., Harvard Med. Sch., Boston, MA

Abstract: Dopamine (DA) neurons in the Ventral Tegmental Area (VTA) and Substantia Nigra (SN) modulate feeding, drinking, and locomotor behaviors, but the signals that regulate DA neurons to dictate behavioral output remain unclear. We examined how the neuropeptide neurotensin (Nts) could modulate DA-mediated behaviors. Nts signals via the G-protein coupled receptors neurotensin receptor-1 (NtsR1) and neurotensin receptor-2 (NtsR2). To determine whether NtsR1 or NtsR2-expressing neurons are present in the midbrain we crossed NtsR1-Cre and NtsR2-Cre mice onto a cre-mediated GFP reporter background, such that only NtsR1 or NtsR2 neurons express GFP and can be visualized. Examination of these mice revealed NtsR1 and NtsR2 neurons in the VTA, most of which also contain tyrosine hydroxylase, and are therefore DAergic. We also identified NtsR1 neurons in the SN, but found essentially no NtsR2 neurons in this brain region. Together, these data suggest that NtsR1 is the predominant receptor isoform in midbrain neurons and is expressed on DA neurons. Since NtsR1 is implicated in the regulation of ingestive behavior and locomotor activity we further hypothesized that disruption of midbrain NtsR1 signaling would impair energy balance. We therefore injected adult NtsR1-Cre mice in the VTA with either a cre-dependent viral vector expressing GFP (generating NtsR1-GFP mice with intact NtsR1 neurons) or the diphtheria toxin subunit A (DTA) to selectively ablate NtsR1 neurons (NtsR1-DTA mice). NtsR1-DTA mice exhibited increased locomotor activity, oxygen consumption and food intake compared to NtsR1-GFP controls. Despite their increased feeding, however, the NtsR1-DTA mice remain leaner than their NtsR1-GFP counterparts, suggesting improved metabolic efficiency. Thus, NtsR1 neurons in the VTA are important for dictating locomotor activity and ingestive behavior, and suggest a role for Nts signaling in modulating DA-mediated behaviors and energy balance.

Disclosures: H.M. Batchelor: None. **H. Woodworth:** None. **J. Brown:** None. **R. Bugescu:** None. **P. Fuller:** None. **G. Leinninger:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.16/BB3

Topic: E.07. Food Intake and Energy Balance

Title: PTP1B deficiency attenuates hypothalamic inflammation through the activation of Jak2-Stat3 signaling pathway downstream of TNFα receptor under high fat diet conditions

Authors: *T. TSUNEKAWA, R. BANNO, M. SUGIYAMA, T. TOMINAGA, T. ONOUE, M. GOTO, H. ARIMA;
Dept. of Endocrinol. and Diabetes, Nagoya Univ. Grad. Sch. of Med., Nagoya, Aichi, Japan

Abstract: Protein tyrosine phosphatase 1B (PTP1B) negatively regulates leptin signaling in the hypothalamus. While a high fat diet (HFD) causes leptin resistance and obesity, whole body or brain specific PTP1B deficiency protects against obesity due to HFD by enhancing leptin sensitivity. PTP1B has also been implicated in the regulation of inflammatory responses in the periphery, while the role of PTP1B in the hypothalamic inflammation under HFD conditions has not been fully elucidated. In the present study, we investigated the effects of PTP1B deficiency on the expression of tumor necrosis factor-alpha (TNFα) and interleukin-10 (IL-10) expression in the hypothalamus. TNFα mRNA expression in the hypothalamus was significantly decreased while IL-10 mRNA expression was significantly increased in the PTP1B whole body knock-out mice (KO) compared to wild-type mice (WT) on HFD at the age of 7 weeks when body weight and visceral fat pad weight were similar between genotypes. While incubation with TNFα significantly increased TNFα mRNA expression in the hypothalamic explants, the expression levels were significantly decreased in KO compared to WT. In contrast, IL-10 mRNA expression was significantly increased with TNFα treatment in the KO hypothalamic explants compared to WT. Incubation with TNFα also increased the phosphorylation of both Jak2 and Stat3 in hypothalamic explants, and the effects were more prominent in KO than in WT, while other signal-transducing protein phosphorylation such as p65, p38, JNK and ERK were similar between genotypes. Incubation with JSI-124 or S31-201, inhibitors of stat3 phosphorylation, suppressed TNFα-induced Stat3 phosphorylation and cancelled the down-regulation of TNFα and up-regulation of IL-10 mRNA expression in KO hypothalamic explants treated with TNFα without affecting other signal-transducing protein phosphorylation such as p65, p38, JNK and ERK. These results suggest that PTP1B deficiency activates Jak2-Stat3 signaling pathways downstream of TNFα receptor, leading to the attenuation of hypothalamic inflammation under HFD conditions.

Disclosures: T. Tsunekawa: None. **R. Banno:** None. **M. Sugiyama:** None. **T. Tominaga:** None. **T. Onoue:** None. **M. Goto:** None. **H. Arima:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.17/BB4

Topic: E.07. Food Intake and Energy Balance

Support: PROMEP

CONACYT

Title: Comparison between water and stevia intake. Expression of IGF-II and its receptor IGF-IIR in Central Nervous System from BALB/c mice

Authors: *E. MORALES, I. CONTRERAS, J. A. ESTRADA;
Univ. Autonoma Del Estado De México, Toluca, Mexico

Abstract: Abstract. Currently, chronic non-communicable pathologies like diabetes mellitus type 2, obesity, hypertension and metabolic syndrome are related to dietary modifications, especially excess intake of carbohydrates, for which alternatives to conserve the sweetness without adding calories have been found in the form of sweeteners, such as esteviol derivatives. Although sweeteners made from esteviol are considered safe, the effects of chronic high-dose intake of these compounds have not been fully determined. The objective of this study was to determine the expression of IGF-II and IGF-IIR in the brain and spinal cord of 8 week old mice supplemented with commercial esteviols for 6 weeks. After supplementation, the brain and spinal cord were used for protein extraction and analyzed by western blot. Our results showed no differences in IGF-II expression in the central nervous system of supplemented mice. An additional band ≥ 250 KDa was found to be expressed differentially in the brain and spinal cord of the animals. It may correspond to the molecule bound to its receptor. We were unable to detect IGF-IIR; however, we found additional bands at ~ 130 KDa, increased in the control group, and another band at ~ 50 KDa, increased in the esteviol group, both from brain tissue. Our data suggest there are no differences in IGF-II or IGF-IIR expression in mice supplemented with esteviols.

Disclosures: **E. Morales:** None. **I. Contreras:** None. **J.A. Estrada:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.18/BB5

Topic: E.07. Food Intake and Energy Balance

Support: National Research Foundation 2013M3C7A1056099

DGIST R&D Program of the Ministry of Science, Information and Communication Technology, and Future Planning 15-BD-0402

Title: Brain-derived insulin is induced by Wnt signaling and secreted in the hypothalamic cells

Authors: *J. LEE[1], K. KIM[1], S.-W. YU[1], E.-K. KIM[1,2];
[1]Dept. of Brain and Cognitive Sci., Daegu Gyeongbuk Instititue of Sci. and Technolo, Daegu, Korea, Republic of; [2]Neurometabolomics Res. Ctr., Daegu Gyeongbuk Inst. of Sci. and Technol., Daegu, Korea, Republic of

Abstract: Insulin plays diverse roles in the brain. Insulin is produced by pancreatic β-cells, and it is a major source of brain insulin, which passes cross the blood-brain barrier. Recent studies support that insulin is produced locally within the brain. However, the mechanisms underlying the production and the distinct function of this brain-derived insulin (BDI) are unknown yet. Here, we examined the effect of Wnt3a, which is involved in the synthesis of insulin in the pancreas, in BDI expression in the hypothalamic cell line. Wnt3a treatment significantly increased the expression of *Ins2* gene, which is the predominant form in the mouse brain, through the activation of Wnt/β-catenin signaling via GSK3β. Moreover, the concentration of insulin in cultured media was higher in Wnt3a-treated group than non-treated group. Interestingly, neurogenic differentiation 1 (NeuroD1), which is a target of Wnt/β-catenin signaling and one of transcription factors for insulin, was also induced by Wnt3a treatment in a time- and dose-dependent manners. Knockdown of NeuroD1 through lentiviral shRNA reduced the basal expression of *Ins2*, and suppressed the Wnt3a-induced *Ins2* gene expression. Taken together, these results suggest that BDI expression is regulated by Wnt/β-catenin/NeuroD1 pathway in the hypothalamus.

Disclosures: **J. Lee:** None. **K. Kim:** None. **S. Yu:** None. **E. Kim:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.19/BB6

Topic: E.07. Food Intake and Energy Balance

Support: R00-DK090101

R01-DK103808

Title: Lateral hypothalamic neurotensin neurons engage the mesolimbic dopamine system to regulate water intake and locomotor activity

Authors: *H. WOODWORTH, H. BATCHELOR, J. BROWN, R. BUGESCU, G. LEINNINGER;
Michigan State Univ., East Lansing, MI

Abstract: The lateral hypothalamic area (LHA) acts in concert with dopamine (DA) neurons in the ventral tegmental area (VTA) to regulate the motivation to feed, drink and move. It remains unclear, however, how specific LHA neuronal populations modify ingestive and locomotor behaviors. We examined how LHA neurons expressing the neuropeptide neurotensin (Nts) engage the DA system to regulate behavior. LHA Nts neurons project to the VTA, where many DA neurons co-express neurotensin receptor 1 (NtsR1). Furthermore, these VTA NtsR1-DA neurons project to ventral striatal brain regions that regulate ingestive behavior and locomotor

activity, such as the nucleus accumbens (NA). Selective activation of LHA Nts neurons using DREADD technology increases pCREB expression in the VTA and NA, confirming that LHA Nts neurons functionally modulate the mesolimbic DA system. Next we examined the physiological role of this circuit by activating LHA Nts neurons in normal mice (WT) and in mice that lack NtsR1 (NtsR1KO mice). Activation of LHA Nts neurons increased locomotor activity and oxygen consumption in WT and NtsR1KO mice, but these effects were blunted by the DA receptor 1 (DR1) antagonist, SCH23390. Acute activation of LHA Nts neurons did not alter chow intake in sated WT mice, but it significantly increased their water consumption. Intriguingly, drinking behavior was not blunted by inhibition of DR1, but was suppressed in NtsR1KO mice, suggesting a specific role for NtsR1 in ingestive behavior. Together, these data reveal that LHA Nts neurons engage the mesolimbic DA system to modify drinking and locomotor behaviors via distinct signaling mechanisms. Intact action via the LHA Nts neuronal circuit is thus crucial for coordinating physical activity and intake of natural rewards, such as water.

Disclosures: H. Woodworth: None. **H. Batchelor:** None. **J. Brown:** None. **R. Bugescu:** None. **G. Leinninger:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.20/BB7

Topic: E.07. Food Intake and Energy Balance

Support: NIH 2T32 DK 064584 (EQC)

NIH NIDDK R01-DK092587 (HM)

NORC P&F P30-DK072476 (HM)

COBRE 1 P20 RR02195 (HM)

Title: Galanin neurons in the lateral hypothalamus modulate locomotor activity

Authors: *E. QUALLS-CREEKMORE, S. YU, C. MORRISON, H. MUNZBERG; Central Leptin Signaling, Pennington Biomed. Res. Ctr., Baton Rouge, LA

Abstract: The lateral hypothalamus (LHA) is an important integrator of reward and appetitive behavior. Previous work in our lab has shown that LHA galanin (Gal) neurons which co-express leptin receptors (LepRb) are implicated in food reward. Using the incentive runway test, we found that mice lacking the leptin receptor on galanin neurons (Gal-LepRbKO) retrieved a palatable treat faster, were less distracted, and showed increased running speed. LHA galanin neurons strongly project to LHA orexin neurons and also to the locus coeruleus, both regions

which are important regulators of reward as well as arousal and locomotor activity. Because locomotor activity is an important component of both feeding behavior and reward, we hypothesized that altered locomotor activity in Gal-LepRbKO mice may contribute to increased performance in the incentive runway test. In this study, we investigated the role of LHA galanin and leptin signaling in locomotor activity. We tested if the increased completion speed of Gal-LepRbKO mice could be attributed to an increase in gross locomotor activity. Gal-LepRbKO show increased gross locomotor activity, which is only significant during the light cycle. Next, we examined the effect of chemogenetic activation of LHA galanin neurons on gross locomotor activity as well as general locomotor activity in the open field test. Here we found that activation of LHA galanin neurons resulted in a robust increase in gross locomotor activity as well as increased distance traveled and average speed in the open field test. Thus, LHA galanin neurons clearly modulate locomotor activity, but the importance of Gal-LepRb neurons vs. the entire population of LHA galanin in select aspects of locomotor activity (e.g. motivational aspect of food reward behavior, general locomotor activity, or stress related activity) remains unclear and is currently under investigation.

Disclosures: E. Qualls-Creekmore: None. **S. Yu:** None. **C. Morrison:** None. **H. Munzberg:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.21/BB8

Topic: E.07. Food Intake and Energy Balance

Support: SEP-CONACYT-Ciencia Básica #220342

UDEM Grant UIN14011

UDEM Grant UIN15027

Title: Prepro-orexin gene promoter is regulated *in vitro* by the transcription factor ebf2 in glial and neural cells

Authors: *R. VIDALTAMAYO[1], A. SÁNCHEZ-GARCÍA[2], R. ORTIZ-LÓPEZ[2], V. ZOMOSA-SIGNORET[2];
[1]Univ. of Monterrey, San Pedro, Mexico; [2]Univ. Autónoma de Nuevo León, Monterrey, Mexico

Abstract: Orexins or hypocretins are neurotransmitters produced by a small population of neurons in the lateral hypothalamus. This family of peptides modulates sleep-wake cycle, arousal and feeding behaviors, and the mechanisms regulating their expression are not completely understood. There is an interest in defining the key molecular elements in orexin regulation, as

these may serve to identify targets to generate novel therapies for sleep disorders, obesity and addiction. We have previously shown that orexin levels decrease in ebf2 KO mice and that the promoter region of the prepro-orexin gene contains two *olf-1* binding sites. Moreover, overexpression of the transcription factor ebf2 induces activity from this promoter *in vitro*, in a non-neural cell line. Here, we analyze the regulation of expression of luciferase driven by the putative promoter region of the murine prepro-orexin gene in lentivirus-transduced C6 glial cells overexpressing ebf2 and in the mHypoA-41 line, that is a cell line derived from male adult C57Bl6 mouse hypothalamic neurons, where we were able to show expression of the ebf2 transcription factor. Both hypothalamic and ebf2-expressing glial cells showed increased luciferase signals, with respect to the original parental C6 line (ANOVA, Bonferroni´s post-hoc test: $P<0.05$). Deletion of the olf1-like proximal to the transcription start site increases the luciferase signal, while the deletion of the distal olf1-like site results in a signal decrease. Mutations of either, or both, *olf1*-like sites do not alter luciferase expression. Our results show that the transcription factor ebf2 can induce gene expression driven by the promoter region of the prepro-orexin gene in different cell lineages.

Disclosures: R. Vidaltamayo: None. **A. Sánchez-García:** None. **R. Ortiz-López:** None. **V. Zomosa-Signoret:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.22/BB9

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DA033811

NSF GRFP

Title: Effects of oral and intragastric glucose delivery on insulin receptor phosphorylation in nucleus accumbens

Authors: *C. WOODS[1], Z. GUTTMAN[2], A. RABINOWITSCH[3], R. KOLARIC[3], K. JONES[3], S. CABEZA DE VACA[3], A. SCLAFANI[4], K. CARR[3];
[2]Ctr. for Neural Sci., [3]Psychiatry, [1]New York Univ., New York, NY; [4]Brooklyn Col. CUNY, Brooklyn, NY

Abstract: Insulin is a metabolic hormone synthesized in beta cells of the pancreas and released upon detection of blood glucose. Insulin actively crosses the blood-brain-barrier and insulin receptors are distributed widely throughout the brain. Insulin activity is dependent upon brain region, and insulin dysregulation in the brain is associated with a variety of disorders. Stouffer et al (presentation, SfN 2012, New Orleans, LA) used fast scan cyclic voltammetry in brain slices

to demonstrate that insulin increases evoked extracellular dopamine concentrations in the Nucleus Accumbens (NAc). The enhancing effect of insulin was greatest in chronically food-restricted (FR), and least in diet-induced obese (OB) rats. In order to translate these findings into an *in vivo* model, we investigated whether consumption of glucose triggers a short latency activation of insulin receptors (IR) in the NAc. Rats that drank 8 ml of 16% glucose during a 7 minute access period displayed increased IR phosphorylation relative to rats with 7 minute access to water. To eliminate the cephalic phase insulin response and achieve precise control over rate and amount of glucose delivery, rats received a 7 minute intragastric (IG) infusion of 8 ml of either 16% glucose or water. IG glucose increased NAc IR phosphorylation at 7 but not 17 or 27 minutes following initiation of infusion. To examine the effect of diet, the experiment was repeated in FR, OB and ad libitum (AL) rats. While both FR and AL rats displayed glucose-induced IR phosphorylation, OB rats did not. These findings parallel the results previously obtained in striatal slices. Finally, in a follow-up behavioral study it was demonstrated that inactivation of insulin, via NAc microinjection of insulin antibodies, interfered with formation of a flavor-glucose preference, while microinjection of IgG did not. Overall, these data suggest that glucose intake triggers a short latency activation of insulin receptors in the NAc which is dependent upon diet and contributes to reinforcement of preference for a flavor that signals a glycemic load. This work was supported by NIH grant DA033811 and the NSF GRFP.

Disclosures: **C. Woods:** None. **Z. Guttman:** None. **A. Rabinowitsch:** None. **R. Kolaric:** None. **K. Jones:** None. **S. Cabeza de Vaca:** None. **A. Sclafani:** None. **K. Carr:** None.

Poster

350. Food Intake and Energy Balance: Neuropeptide Regulators

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 350.23/BB10

Topic: E.07. Food Intake and Energy Balance

Support: RASLR 7, 2007 (CRP-59764-F71J12000990002)

Title: Orexin A administration by lactoferrin- and antitransferrin-modified liposomes potentiate the nucleus accumbens shell dopamine responsiveness to food

Authors: *V. BASSAREO[1], F. CUCCA[2], R. FRAU[2], F. LAI[3], F. CORRIAS[3], A. M. FADDA[3], G. DI CHIARA[2];
[2]Biomed. Sci., [3]Life and Enviromental Sci., [1]Univ. of Cagliari, Cagliari, Italy

Abstract: Orexin neurons originate in the hypothalamic region and project to different brain areas. They produce two different neuropeptides: orexin A (Orx A) and orexin B (Orx B) and two receptors for the orexin system has been characterized: OxR1 and OxR2. OxR1 binds orexin A with 30 nM affinity but has much lower affinity for orexin B, whereas OxR2 binds both orexin

peptides with similar high affinity. Several studies reported that orexinergic neurons in the lateral hypothalamus (LH) are involved in motivated behavior for drugs of abuse as well as natural rewards. In particular administration of orexin A has been shown to stimulate food consumption, and orexin signaling in VTA is implicated in intake of high-fat food. It has been shown that LH orexin neurons project to the ventral tegmental area (VTA) indicating that the VTA is an important site of action for orexin's role in reward processing. Mesolimbic dopamine (DA) in the nucleus accumbens (NAc) shell is also involved in the rewarding mechanisms of food consumption. Is there a cooperation between DA and Orx A? In our study we investigated by brain microdialysis the responsiveness of NAc shell DA transmission in food consumption after intravenous administration of Orx A encapsulated in different targeted and not-targeted stealth liposomes prepared using film hydration method. Orx A per se produced a later increase of DA in the NAc shell (peaking at 80 min sample) and strengthened the DA responsiveness in this area after sucrose pellets feeding. We registered also an increase of the number of eaten pellets. These effects on DA and on feeding were blocked by intraperitoneally injection of the antagonist of the OxR1 (SB 334867, 30mg/Kg). We can speculate that the strengthening of DA response during food consumption exerted by Orx A administration could increase the rewarding properties of food and could be one of the mechanisms that underlie food addiction. These findings could suggest new targets for a new treatment of eating disorders.

Disclosures: **V. Bassareo:** None. **F. Cucca:** None. **R. Frau:** None. **F. Lai:** None. **F. Corrias:** None. **A.M. Fadda:** None. **G. Di Chiara:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.01/BB11

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 82638

CIHR Grant 86727

CIHR Grant 230771

Title: Hippocampus and cortical plasticity following a virtual spatial memory intervention program promote spontaneous hippocampus-dependent navigation strategies in healthy older adults

Authors: *D. DUCHARME[1], D. SODUMS[1], K. KONISHI[1], L. DAHMANI[1], L. BHERER[2], V. D. BOHBOT[1];
[1]Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada; [2]CRIUGM, Dept. of Psychology, Univ. of Montreal, Montreal, QC, Canada

Abstract: Aim: Studies conducted in our laboratory indicate that healthy individuals initially navigate virtual environments by using one of two spontaneous strategies: the hippocampus (HPC)-driven spatial memory strategy or the caudate nucleus-mediated stimulus-response strategy. The use of these strategies has been linked to increased functional activity and grey matter in their respective brain loci. Because the HPC is affected in both function and volume during normal aging, we created a computer-based spatial memory intervention program (SMIP) specially designed to stimulate this area by promoting the use of spatial memory strategies. Methods: Twenty one healthy older adult participants attended 16 1-hour sessions twice a week over the course of 8 weeks where they learn the relative positions of objects, landmarks, or rooms in virtual environments. Participants underwent a Magnetic Resonance Imaging scan before and after the SMIP as well as multiple virtual navigation tasks such as the 4-on-8 virtual maze to assess potential changes in spatial memory. The 4-on-8 virtual maze is a task that requires remembering the locations of 4 objects in an 8-arm radial maze. A probe trial whereby all landmarks are removed allows the discrimination of spatial versus response spontaneous navigational strategies because errors are made only in people who used landmarks. Results: After intervention, 11 of the 21 participants showed a significant increase in grey matter in the right posterior HPC. When comparing the responders (those who showed grey matter increase) and non-responder groups, it was found that responders had more probe errors on the 4on8 virtual maze ($p<0.05$). Furthermore, increases in grey matter positively correlated with number of errors during the probe trial ($r=0.78$, $p<0.001$), which is indicative of using a spatial strategy to learn the task. Conclusion: These results indicate the potential efficacy of a spatial memory intervention program at facilitating the spontaneous use of hippocampus-dependent spatial navigational strategies over stimulus-response strategies. Our results also show that the degree of increase in hippocampal grey matter following training is directly related to the extent of spatial memory strategy use.

Disclosures: **D. Ducharme:** None. **D. Sodums:** None. **K. Konishi:** None. **L. Dahmani:** None. **L. Bherer:** None. **V.D. Bohbot:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.02/BB12

Topic: F.01. Human Cognition and Behavior

Support: FRQNT-Team Grant # 181390 (G.W., P.J., V.B)

NSERC Discovery Grant # 436140-2013

FRQNT-Early Researcher Grant # 181515 (G.W.)

Title: Landmark use during navigation is associated with increased visual attention to peripheral targets

Authors: B. DRISDELLE[1], K. KONISHI[3], P. JOLICOEUR[1], V. BOHBOT[3], *G. WEST[2,1]; [1]Univ. of Montreal, Montreal, QC, Canada; [2]Psychology, Univ. of Montreal, Outremont, QC, Canada; [3]McGill Univ., Montreal, QC, Canada

Abstract: Aim: When navigating, people adopt different strategies. The spatial strategy involves building relationships between landmarks in an environment to create a cognitive map. The response strategy, in contrast, entails learning a series of movements (e.g., left and right turns) from given positions that act as stimuli. The current study investigated whether different navigation strategies are associated with differences in visual attention performance during a target detection task. Methods: We measured participants' navigation strategies (response vs. spatial), using the 4-on-8 virtual maze (4/8VM). The 4/8VM consists of an 8-arm radial maze, in which 4 arms are accessible and 4 are blocked. Participants have to retrieve objects located at the end of the 4 accessible arms. Then, all 8 arms become accessible and participants have to retrieve objects now located in the 4 arms that were previously blocked. After participants have learned the task, a probe trial is given in which all landmarks are removed. The probe trial allows to dissociate spatial and response learners, as spatial learners make more mistakes compared to response learners Participants were divided into two groups based on their probe score which indicated those who used landmarks (n = 37) and those that did not (n = 16) during navigation. To measure differences in visual attention performance between both groups, we employed an ERP target detection task that was designed to elicit a robust ERP component, the N2pc (N-2-posterior-controlateral), a lateralized component thought to be modulated by target selection and distractor inhibition in the visual domain. The task had both central and peripheral target conditions. Results: Our results revealed a robust correlation between N2pc amplitude produced by peripheral targets and 4/8VM probe errors. Further, when comparing the N2pc amplitude of both groups, a main effect was found where the landmark group produced a larger N2pc than the no-landmark group. Corrected post-hoc comparisons confirmed that this difference was driven by the peripheral target trials. Conclusion: Our results indicate that individuals who rely on landmarks during navigation show increased deployment of visual attention to peripheral targets.

Disclosures: B. Drisdelle: None. **K. Konishi:** None. **P. Jolicoeur:** None. **V. Bohbot:** None. **G. West:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.03/BB13

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 82638

CIHR Grant 86727

CIHR Grant 230771

Title: Selective increase in hippocampus grey matter following a virtual spatial memory intervention program is associated with corresponding memory gains in healthy older adults

Authors: *D. SODUMS[1], K. KONISHI[1], L. DAHMANI[1], L. BHERER[2], V. BOHBOT[1]; [1]Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada; [2]CRIUGM, Dept. of Psychology, Univ. of Montreal, Montreal, QC, Canada

Abstract: Aim: Different navigation strategies dependent on separate memory systems are available to people when they navigate in a virtual environment. Specifically, the use of spatial memory strategies is associated with increased activity and grey matter in the hippocampus (HPC) while the use of stimulus-response strategies is associated with increased activity and grey matter in the caudate nucleus. Since healthy aging affects the function and volume of the HPC, we developed a computerized spatial memory intervention program (SMIP) to specifically stimulate this region. This program is designed to promote the use of spatial memory strategies, taking particular attention to avoid use of stimulus-response strategies Methods: Healthy older adult participants (n=21) underwent the SMIP or control condition (n=33). Each participant underwent a Magnetic Resonance Imaging scan before and after the SMIP and performed independent virtual navigation tasks, such as the Concurrent Spatial Discrimination Task (CSDLT), 4on8 virtual maze, and Go/No-Go task to assess changes in spatial memory. The CSDLT and Go/Go-No tasks are virtual 12-arm radial mazes in which participants have to learn the location of objects within 6 pairs of arms. The 4 on 8 virtual-maze is a task that requires remembering the locations of 4 objects in an 8-arm radial maze. VBM was used to regress grey matter increases with performance improvements on the pre/post intervention tasks. Results: After the SMIP, there was an increase in grey matter throughout cortex, including the bilateral HPC. Participants were categorized as responders (n=11) or non-responders (n=10) depending on whether they showed a significant increase in grey matter. In the responder group, the area with greatest increase in grey matter was the right posterior HPC. When correlating the amount of change in this region with the amount of change in navigation performance from pre- to post-training in all 21 participants, we found that increases in grey matter correlated with fewer trials needed to reach criterion on the 4-on-8 virtual-maze following the training program. There was a significant negative correlation between increase in grey matter and time spent time spent finding all the target objects in both the CSDLT and Go/No-Go tasks, following the SMIP. Conversely, those who did not show increases in grey matter showed no memory gains on the pre/post intervention tasks. Conclusion: These results show that increased grey matter of the HPC is associated with improved memory gains, on tasks independent of the SMIP. These findings suggest that the SMIP is effective at reducing memory deficits associated with loss of HPC grey matter during aging.

Disclosures: D. Sodums: None. **K. Konishi:** None. **L. Dahmani:** None. **L. Bherer:** None. **V. Bohbot:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.04/BB14

Topic: F.01. Human Cognition and Behavior

Support: NIH (R01-NS074450)

Title: Multivariate pattern analysis of human intracranial electrocorticography predicts location in a virtual spatial navigation task

Authors: *A. A. ROBBINS[1], P. HORAK[2], A. CONNOLLY[2], B. JOBST[2];
[1]Dartmouth Neurol., Lebanon, NH; [2]Neurol., Geisel Sch. of Med., Lebanon, NH

Abstract: Spatial navigation tasks are often used in experimental neuroscience to investigate the mechanisms of learning and memory. These tasks provide insight into the dynamics of the brain processes involved in memory tasks. In this study we recorded intracranial EEG (iEEG) from depth and strip electrodes that were implanted in patients with intractible epilepsy while they performed a spatial working memory task. We designed a virtual T-maze memory task (vTMT) for this purpose using the Valve™'s Source Engine. When performing the task, the subjects learn to travel to alternating sides of the maze in order to collect rewards. The vTMT has three levels, which differ in the number visual cues available to distinguish the two sides of the maze. iEEG data were collected at 1600Hz and down-sampled to 200Hz. We calculated a Morlet wavelet transform on 60 log-spaced frequency bands from 2-100Hz to determine the power and phase of each frequency band. The power data were used to train a linear discriminant analysis (lda) classifier to predict whether subject were in the central corridor or an arm of the maze. We were able to predict virtual locations with above chance accuracy ($p<0.05$, student's t-test) in a cohort of 5 subjects. We previously reported that beta power in the hippocampus is higher in the central corridor than in the choice arms of the vTMT. This analysis provides additional evidence that it is possible to distinguish the location of subjects in a virtual environment from iEEG recordings.

Disclosures: **A.A. Robbins:** None. **P. Horak:** None. **A. Connolly:** None. **B. Jobst:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.05/BB15

Topic: F.01. Human Cognition and Behavior

Support: ScienceCampus Tuebingen

Title: The neural correlates of arithmetic learning in children: A fNIRS study

Authors: *M. SOLTANLOU[1,2,3], C. ARTEMENKO[4], T. DRESLER[4,5], F. HAEUSSINGER[5], S. HUBER[3], A. J. FALLGATTER[5,4], A.-C. EHLIS[5,4], H.-C. NUERK[1,3,4];
[1]Dept. of Psychology, Eberhard Karls Univ., Tuebingen, Germany; [2]Grad. Training Ctr. of Neuroscience/ IMPRS for Cognitive and Systems Neurosci., Tuebingen, Germany; [3]Knowledge Media Res. Ctr., Tuebingen, Germany; [4]LEAD Grad. School, Eberhard Karls Univ., Tuebingen, Germany; [5]Univ. Clin. of Psychiatry and Psychotherapy, Dept. of Gen. Psychiatry, Eberhard Karls Univ., Tuebingen, Germany

Abstract: Arithmetic training can improve the mathematics competence. For multiplication, this competence increase is reflected in a shift from effortful processing to fast retrieval, which is accompanied by specific changes in brain activation patterns. Studies in adults show less activation in frontal areas such as the inferior frontal gyrus and parietal areas including the intraparietal sulcus and the inferior parietal lobe. Stronger activation is found in the left angular gyrus. However, children's brain functioning in numerical tasks differs from that of adults. In the current study, we investigated interactive multiplication learning in children to examine if they rather reflect similar changes as in development or learning effects as observed in adults. 24 children from grade 5 (age between 10 and 12 years) were measured using functional near-infrared spectroscopy (fNIRS) before and after multiplication training. In a within-subject design four different conditions were investigated: trained simple, trained difficult, untrained simple, untrained difficult. Children were trained on a simple and difficult set (8 trials each), while they did not receive any training for two other matched sets of multiplication. Children underwent seven training sessions over the course of two weeks. Behavioral results consist of number of correct responses and reaction time. Dependent t-test analyses of correct responses and RTs, separately, revealed that children improved significantly in trained simple, trained difficult, and untrained difficult; but not in untrained simple condition after the training. FNIRS results showed that after these sessions the trained difficult condition now indicated notably less frontal and parietal engagement, especially right. However, the engagement of involved brain areas in trained simple and in both untrained conditions did not differ considerably. Moreover, post-training contrast (trained versus untrained) revealed significantly less frontal and parietal engagement in the difficult, but not in the simple condition. Unexpectedly, stronger left angular activation was not found in the present study. The findings point to the idea that multiplication learning in children may not involve only a strategy shift such as in adults, but also reflect a facilitation of a developmental maturation process.

Disclosures: **M. Soltanlou:** None. **C. Artemenko:** None. **T. Dresler:** None. **F. Haeussinger:** None. **S. Huber:** None. **A.J. Fallgatter:** None. **A. Ehlis:** None. **H. Nuerk:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.06/BB16

Topic: F.01. Human Cognition and Behavior

Support: The I-CORE program of the Planning and Budgeting Committee and The Israel Science Foundation (grant No. 51/11)

Title: Brief episodes of memory reactivation enable perceptual learning

Authors: *R. AMAR[1], S. NEMNI[2], N. CENSOR[1,2];
[1]Sch. of Psychological Sci., [2]Sagol Sch. of Neurosci., Tel Aviv Univ., Tel-Aviv, Israel

Abstract: Perceptual learning refers to improvements in perception thresholds with practice. Previous studies have shown that following initial encoding and consolidation of the perceptual memory, perception thresholds can continue to gradually improve over multiple training sessions, similar to other forms of procedural learning. Thus in the context of procedural learning, consolidation refers not only to stabilization of the memory but also to improvements in performance that occur after the end of practice, referred to as offline gains (Censor, Sagi and Cohen, 2012). Here we show that following initial training, brief sessions in which the encoded visual memory is reactivated, are sufficient to enable perceptual learning comparable to learning achieved with repeated standard practice sessions. Participants trained with the texture discrimination task (Karni and Sagi, 1991). The texture stimulus was presented for 10ms, followed by a patterned mask which was presented for 100ms. Observers decided whether an array of 3 diagonal bars embedded in an array of horizontal bars (19×19) was horizontal or vertical. The target-to-mask asynchrony (SOA) was randomly changed within the session (14 SOAs ranging between 40ms and 340ms, 18 trials per SOA, 252 trials total) to obtain a psychometric curve, from which the SOA discrimination threshold was derived. The memory was first encoded and consolidated following a full session on Day 1. Then, participants returned for three daily reactivation sessions (Days 2-4) of only 5 trials each, at a near-threshold SOA (derived from Day 1). On Day 5, participants returned for a full test session (identical to Day 1 session). Learning, measured as improvement in discrimination thresholds from Day 1 to Day 5, was significant and comparable to total Day 1 to Day 5 learning in a control group of subjects performing 5 regular full daily sessions. The control group showed standard gradual threshold improvements over the course of 5 days. These results suggest that brief reactivations of consolidated perceptual memories may enable efficient perceptual learning, possibly via reactivation-reconsolidation cycles of memory strengthening (Lee, 2008; Dudai, 2012; Nader and Hardt, 2009). This interpretation may have an important role in understanding the mechanisms underlying perceptual learning, and enable the development of novel strategies geared to substantially reduce the amount of practice needed to enable learning in normal conditions and following neurological diseases or brain injuries.

Disclosures: **R. Amar:** None. **S. Nemni:** None. **N. Censor:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.07/BB17

Topic: F.01. Human Cognition and Behavior

Support: NSERC

Title: Women and men use different default spatial strategies to solve a real world navigation task

Authors: ***M. FIDA**, E. L. ZELINSKI, R. J. SUTHERLAND;
CCBN, Lethbridge, AB, Canada

Abstract: Sex differences in spatial behavior have been reported in many mammalian species, including humans. The Morris Water Task (MWT) is an often-used behavioral assay of spatial ability in rodents that has been adapted to use in humans, typically as virtual reality or tabletop versions. Such variations have lead some to hypothesize that males and females implement different strategies to solve spatial problems. Typically, men use cardinal directions, whereas women use landmarks. However, it could be the case that peri-personal tasks recruit different neural systems than would be engaged during large-scale, real-world traversals. Thus, we developed a dry-land version of the MWT wherein subjects were required to traverse a circular, outdoor area (diameter: 20-meters). We hypothesized that men and women (aged 19-25) would implement different strategies to solve the task. Forty-four subjects (27 women) were asked to locate a single, hidden target location over several trials with varying start locations. Both sexes reached the same level of performance by the end of training, but the results demonstrated that men and women use, as a default, allocentric and egocentric strategies, respectively. A second cohort containing seventeen subjects (13 women) performed the task, but the starting location remained constant for the first and second trials. In the second condition, women proceeded directly to the platform location on the second trial. Together, these results indicate that although men and women can both solve spatial tasks, the default strategy is allocentric for men and egocentric for women.

Disclosures: **M. Fida:** None. **E.L. Zelinski:** None. **R.J. Sutherland:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.08/BB18

Topic: F.01. Human Cognition and Behavior

Support: NSERC

AIHS

CFI

Title: Behavioural and electrophysiological characteristics of virtual navigation task performance in men and women

Authors: *E. L. ZELINSKI, M. FIDA, R. J. SUTHERLAND;
Neurosci., Canadian Ctr. For Behavioural Neurosci., Lethbridge, AB, Canada

Abstract: Examination of the neurological underpinnings of spatial abilities often requires human subjects to be stationary. Thus, virtual reality is a commonly used tool for elucidating how we process information about the world around us. We hypothesized that differences in navigational skill would impact the strategy implemented by subjects, whether they would notice changes to the environment, and the electrophysiological responses to the presentation of manipulated vs. non-manipulated and remembered, non remembered, and novel scenes during performance of a virtual navigation task. Object-location memory was assessed within a large-scale, virtual environment whilst recording dense array EEG. Subjects were restricted to a central circular area by a fence, but the world beyond (e.g., mountains, seascape, forest) remained visible. In addition to these distal cues, various naturalistic objects (e.g., boulders, trees) were present within the navigable portion of the environment to provide proximal cues. During the exploration phase of the task, subjects moved through the environment until all object-location pairs were encountered. During the second phase of the task, subjects were shown pictures of the objects with background images that were either congruent with the original object-location pair or incongruent. Subjects began each trial at a central start location and rotated until their perceived optimal approach angle followed by direct traversal to the current trial target location. The final phase of the task required subjects to place objects in their original locations over a topographical representation of the environment. Several factors influence performance including sex and experience playing video games.

Disclosures: E.L. Zelinski: None. **M. Fida:** None. **R.J. Sutherland:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.09/BB19

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant EY017605

Title: Transcranial alternating current stimulation strengthens learning of color-orientation associations

Authors: *Y. LIU[1,2], K. KAR[1,2], B. KREKELBERG[1];
[1]Ctr. for Mol. and Behavioral Neurosci., [2]Behavioral and Neural Sci., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Transcranial current stimulation (tCS) has been found to improve cognitive function in various domains (e.g. memory, language, vision, cognitive aging, etc.). Despite its growing use, the underlying neural mechanism remain unclear. To investigate how tCS influences learning, we examined the effects of transcranial alternating current stimulation (tACS) on the McCollough Effect (ME). In contrast to many visual aftereffects that typically extinguish within a few seconds, the ME is an orientation-contingent color aftereffect that can persist for days. This long time scale as well as the orientation contingency of the ME indicate that the acquisition of the ME can serve as a low level model of a learning process that associates orientation and color in the visual pathway. Moreover, there is strong evidence to suggest that the ME originates in primary visual cortex (V1). These properties make the ME a suitable phenomenon to study the neural basis of tACS' influence on learning. We induced the ME in participants with two alternating sets of gratings paired with two complementary colors (vertical-red and horizontal-green, alternated every 1 s for 4 s: induction stimuli). After induction, a test pattern (300 ms) of neutral white gratings was perceived to be tinted with colors complementary to the original pairing (i.e., vertical-green and horizontal-red). We quantified this ME by adjusting the colors of the test patterns to null participants' perceived ME. This procedure was repeated to build up the strength of the ME. In the tACS condition, electrical stimulation (0.5 mA, 50 Hz) was applied with one electrode over area V1 (Oz) and one on the vertex (Cz). Stimulation was only applied during the presentation of the induction stimuli. In the absence of tACS, the build-up of the ME typically saturated within a few minutes. In the presence of tACS, however, no such saturation was observed. As a consequence, the ME was significantly larger in the tACS compared to the no-tACS condition. In other words, tACS strengthened the learning of color-orientation association that is necessary for perceiving the ME. Based on our previous work (Kar and Krekelberg, J. Neurosci. 34.21 (2014): 7334-7340), we speculate that tACS attenuated the adaptation of early visual neurons sensitive to orientation or color. This reduced adaption would result in stronger input to neural populations that detect the conjunction of orientation and color, and this could enhance the learning of the orientation-color association. These results lay the groundwork for future experiments that will test this hypothesis using behavioral experiments and electrophysiological recordings in macaque V1.

Disclosures: Y. Liu: None. **K. Kar:** None. **B. Krekelberg:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.10/BB20

Topic: F.01. Human Cognition and Behavior

Support: HHMI Undergraduate Grant # 52006934

Title: Learning on a musical-interval discrimination task through a combination of task practice and stimulus exposure alone

Authors: *D. F. LITTLE, H. CHENG, B. A. WRIGHT;
Communication Sci. and Disorders, Northwestern Univ., Evanston, IL

Abstract: Introduction A fundamental component of musical skill is the ability to discriminate between and identify musical intervals, fixed ratios between pitches. Learning this skill is challenging because the same musical interval can be instantiated by multiple pairs of pitches, and even identification of a single instance of an interval is difficult. Here we asked whether a training regimen that combined periods of task practice with periods of stimulus-exposure alone facilitates musical-interval learning. Similar regimens have been particularly effective at inducing learning on basic discrimination tasks in audition and vision. These outcomes are of particular interest because the practice-plus-exposure combination can yield learning even when neither element does so in isolation. If this regimen yields musical-interval learning, it would indicate that it aids discrimination as well as category formation. **Methods** In four different 4-day training regimens, each day was divided into four ~8 minute blocks of either practice, stimulus exposure, or silence. During practice blocks, listeners discriminated multiple instances of perfect 4ths from major 3rds, perfect 5ths, and major 6ths, for 60 trials. During exposure blocks, listeners performed a written matching task while 60 examples of perfect 4ths were presented in the background. During silence blocks, listeners performed a mock musical-interval discrimination task in silence. On each day, listeners (n=7 per group) received either (1) 180 stimulus exposures (Exposure+Silence), (2) 180 practice trials (Practice+Silence), (3) 360 practice trials (All Practice) or (4) 180 trials + 180 exposures (Practice+Exposure). **Results** Discrimination accuracy improved by ~24 percentage points with the Practice+Exposure regimen, compared to ~11 to ~14 percentage points for the Exposure+Silence, Practice+Silence and All-Practice regimens. The improvement was greatest for the Practice+Exposure regimen (all $p < 0.001$), and did not differ significantly among the remaining three regimens ($p \geq 0.934$). The benefits conferred by the Exposure+Practice regimen generalized to untrained stimuli (triangle tones) for the trained task and to an untrained task (interval identification) for the trained musical interval (the 4th). **Conclusions** Pairing practice with stimulus-exposure alone yielded learning on a musical-interval discrimination task, extending the demonstrated benefits of this regimen beyond basic discrimination tasks to a challenging case requiring category formation that is of practical use. This result suggests that a similar mechanism drives the observed enhancements to learning in both cases.

Disclosures: **D.F. Little:** None. **H. Cheng:** None. **B.A. Wright:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.11/BB21

Topic: F.01. Human Cognition and Behavior

Title: The impact of olfactory stressors on perceptual recall and timing behavior

Authors: *J. E. WILLIAMS, M. A. WILLIAMS;
Dept. of Psychology, Eastern Illinois Univ., Charleston, IL

Abstract: This experiment extends our previous work testing people's ability to correctly perceive the amount of time that occurs as well as amount of information recalled during the presentation of differing suspenseful endings to a video which may or may not lead to a stress response. It was previously shown that auditory and visual stimuli significantly reduced recall and increased judgements of passage of time. It was therefore hypothesized that olfactory factors related to the body's response in stressful situations would also cause impairment of the ability to determine correctly how long the presentation was, and reduce total recall of events, especially for the group viewing the video with higher stress inducing content, i.e., the more stressful film ending, in conjunction with a noxious odor. 137 undergraduate subjects provided informed consent, were presented with a 3-5 minute video clip from a recent movie title "Hide and Seek", and then completed survey questionnaires about the video along with two survey components which measured the subjects vulnerability to stress and the extent to which they experience stress related symptoms in their life. Each of the subject groups viewed one of two alternate endings to the movie not presented in the original release of the movie. One group was shown a happy or "non-menacing" clip and another group was shown a clip containing "menacing" content believed to induce a stress response. The groups were tested with the addition of either a eucalyptus odor, or a deer repellant (rotten egg/urine) odor. Results suggest that while physical stressors (sights, sounds), and psychologically charged material cause impairment of recall and perception, the presentation of olfactory stimuli also leads to significant increases in perception of time passage and a decrease in total recall. Data are discussed relevant to the ability of emergency responders to perform during high stress context situations. The possibility exists that professional responding during emergency situations is differentially affected by context.

Disclosures: **J.E. Williams:** None. **M.A. Williams:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.12/BB22

Topic: F.01. Human Cognition and Behavior

Title: Perceptual learning for detection of multiple features produces non-independent processing: Behavioral and neurophysiological evidence

Authors: *M. J. WENGER[1,2], S. E. RHOTEN[1];
[1]Ctr. for Applied Social Res., The Univ. of Oklahoma, Norman, OK; [2]Div. of Nutritional Sci., Cornell Univ., Ithaca, NY

Abstract: We previously (SfN 2014) reported EEG and behavioral data (response frequencies) supporting the hypothesis that that perceptual learning can be obtained with multiple features and that learning involves the development of non-independence at perceptual and decisional levels. This is consistent with evidence suggesting that multiple levels of processing and representation may be involved in perceptual learning. The present study explores these notions using EEG and response times (RTs), testing the hypothesis that learning of multi-feature patterns results in processing that is non-independent and parallel, as would be expected if the stimuli were learned as integral objects. Stimuli contained contrast-defined features, extracted from the 1865 drawing of the Cheshire Cat in Alice's Adventures in Wonderland. Participants began by performing a detection task implemented as a double-factorial paradigm (DFP), in which stimuli contained 0, 1, or 2 targets, at two levels of contrast, and were instructed to give a positive response if they judged the stimulus to have either 1 or 2 targets. They then practiced with all possible stimuli for 10-15 days, using an adaptive staircase procedure to track thresholds. Finally, they again performed the DFP detection task with stimuli presented at threshold and the two initial levels of contrast. EEG data were collected during both pre- and post-practice performance of the DFP. Analysis of the RT data, at both the level of the mean and the distribution, suggested that the stimuli were initially processed serially, with practice resulting in a transition to dependent parallel processing and modulations of amplitudes of early ERP features, pre- and post-stimulus spectral power, and reductions in post-stimulus γ−band power, selective to frontal electrodes. These results are consistent with the idea that perceptual learning of multi-feature patterns produces shifts in fundamental characteristics of processing consistent with the learning of an integral object.

Disclosures: M.J. Wenger: None. **S.E. Rhoten:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.13/BB23

Topic: F.01. Human Cognition and Behavior

Support: St. Olaf College

Title: Long-term high-variability training for new adult cochlear implant users

Authors: *J. L. LOEBACH;
Psychology, St. Olaf Col., Northfield, MN

Abstract: When an individual receives a cochlear implant (CI) as a treatment for deafness, they undergo an intense period of perceptual learning to be able to hear with their prosthesis. However, the process of perceptual learning is not fully understood, and there is much debate regarding the types of materials that training should include, and how well they will generalize to novel tasks. An analytic approach to training focuses on phoneme discrimination with the hopes that it will generalize to sentences and real world listening contexts. A synthetic approach focuses on understanding the meaning or gist of the sentence, in hopes that it will generalize to phoneme identification and real world listening situations. While both approaches can yield successes, they tend to be limited to similar conditions that were experienced during training, and may not generalize broadly to new materials and environments. In addition, postlingually deafened adult cochlear implant users generally do not receive any formal training after their implants are activated. As a result, there is a great deal of variability among individual CI users in terms of their satisfaction, performance and comfort in using their implant. It is possible that the lack of training yields to the development of vastly different neurocognitive mechanisms across users, contributing to the variability in satisfaction and use and possibly limiting their performance in real world listening situations. A main objective of our research is to develop a long-term high-variability training program to help new adult cochlear implant users learn to hear with their devices. Utilizing tasks that engage higher-level language (meaningful and anomalous sentences), lower level linguistic (phoneme discrimination) in addition to nonlinguistic speech (talker identification) and general auditory tasks (environmental sound identification), this program trains neurocognitive systems that overlap between linguistic and nonlinguistic systems. This poster will present an overview of the program, including data from normal hearing listeners who helped develop and pilot the project, experienced adult CI users who provided the baseline measurements so that we can build a set of expectations for performance after training, and new CI users who have tested the program over of a 1 month period. Since most adult CI users to not undergo any formal training or rehabilitation, our findings have both theoretical and clinical significance. The results will be discussed in terms of perceptual learning, and interactions between general auditory and speech specific cognitive abilities.

Disclosures: J.L. Loebach: None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.14/BB24

Topic: F.01. Human Cognition and Behavior

Support: NSF REAL Grant 1420211

NSF GRFP Grant DGE-1256259

Title: Grounding symbolic fractions in the ratio processing system: A developmental fMRI-A study

Authors: *E. Y. TOOMARIAN, M. R. LEWIS, J. V. BINZAK, E. M. HUBBARD;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Children and adults experience difficulties when attempting to understand fractions, leading some theorists to propose that fractions might lack a cortical specialization analogous to the Approximate Number System. However, emerging data have suggested that newly identified circuits may be ideally suited for learning about fractions (Lewis, Matthews and Hubbard, in press). This system, known as the Ratio Processing System (RPS), represents the magnitudes of nonsymbolic ratios such as the relative lengths of two lines. Previous single-unit physiology studies in non-human primates and fMRI studies in humans have demonstrated that nonsymbolic line ratios and symbolic fractions elicit responses in regions of the intraparietal sulcus (IPS) and prefrontal cortex (PFC) (Jacob, Vallentin & Nieder, 2012). However, none of these previous studies have tested a critical prediction of the RPS theory: the same neural systems that support nonsymbolic ratio processing should also support symbolic fraction processing, and should do so in a semantically meaningful way. To test whether symbolic fractions are grounded in the RPS, we used an fMRI adaptation (fMRI-A) paradigm in a developmental sample to demonstrate cross-notation recovery from adaptation to nonsymbolic ratio magnitudes. During fMRI scanning, participants were adapted to a specific non-symbolic ratio magnitude- a series of line ratios in which the component line lengths varied but the shorter line length was always the same fraction of the longer line, i.e., 3/10 or 7/10 across runs. This was followed by presentation of either symbolic or non-symbolic deviant magnitudes, either close to or far from the adapting value. Deviants that were close to 3/10, like 2/9, were numerically distant from 7/10, and deviants that were numerically close to 7/10, like 7/9 were far from 3/10. Activation in right mid-IPS recovered in a distance-dependent fashion (greater response for far deviants than close deviants) when either a nonsymbolic ratio or corresponding symbolic fraction was presented. The fact that adaptation transferred from nonsymbolic line ratios to symbolic fractions in a distance-dependent manner suggests that symbolic fractions are semantically mapped to more basic RPS represention of nonsymbolic ratio magnitude. We are currently exploring how these mechanisms change with development during the acquistion of symbolic fraction knowledge during the early school years. A better understanding of these brain systems that support ratio processing may lead to improved educational methods for fractions.

Disclosures: **E.Y. Toomarian:** None. **M.R. Lewis:** None. **J.V. Binzak:** None. **E.M. Hubbard:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.15/BB25

Topic: F.01. Human Cognition and Behavior

Support: DFG SFB 874

International Graduate School of Science (Ruhr-Universität Bochum) Stipend for AM

Title: Orientation discrimination performance improves after "LTP-like" visual stimulation applied via Oculus Rift video device

Authors: ***A. MARZOLL**[1], E. KEßEL[1], H. R. DINSE[1,2];
[1]Inst. für Neuroinformatik - Neural Plasticity Lab., Ruhr-Universität Bochum, Bochum, Germany; [2]Neurologische Klinik am Berufsgenossenschaftlichen Universitätsklinikum Bergmannsheil, Ruhr-Universität Bochum, Bochum, Germany

Abstract: Recently, it was shown that sensory performance in the tactile and visual domain could be selectively enhanced or impaired by employing peripheral stimulation protocols (Ragert et al., 2008; Beste et al., 2011). The temporal properties of these protocols mimicked those used to induce Hebbian forms of plasticity in cellular studies and were thus dubbed "LTP-like" and "LTD-like". Here, we investigated whether it is possible to improve perceptual performance in a visual orientation discrimination (OD) task by administering visual LTP-like stimulation. To find an innovative and simple way to apply visual stimulation concurrent to normal viewing, subjects wore a head-mounted display device (Oculus Rift DK2, Oculus VR®) for three hours while either LTP-like or sham stimulation were presented superimposed on a movie. LTP-like stimulation consisted of a rectangular field of sinusoidal gratings flickering intermittently between two orientations (30° and 120° at 1 cpd) for 5 s duration and a subsequent period of 5 s where no gratings were displayed, for 1080 stimulation cycles per subject. Sham stimulation for the control group consisted of static display (30 s periods) of the same gratings, matching the total display duration of LTP-like stimulation. For assessment of OD performance, before and after stimulation subjects performed a visual OD task for the two stimulated orientations (30°/120° at 1 cpd). As control conditions, one of the stimulated orientations was tested at another spatial frequency (30° at 2 cpd), and a non-stimulated orientation was tested (75° at 1 cpd). Subjects had to indicate whether a Gabor-patch deviated from the target orientation in a clockwise or counter-clockwise manner. Participants who were subjected to LTP-like stimulation improved their OD thresholds specific to the orientations presented during stimulation by, on

average, more than 10%. No such improvement happened for the control orientation (75°) or in subjects receiving sham stimulation. Our data demonstrate that passive visual stimulation can be used to induce plastic changes in an OD task specific to the parameters of repetitive stimulation. Unlike previous studies in the visual domain, which investigated the influence of stimulation on attentional processes (Beste et al., 2011), here we could show improved discrimination ability for a low-level visual feature, suggesting an enhanced representation. Stimulation via head-mounted devices promises to be viable and practical means of inducing changes of visual perception, which might be of considerable benefit in conditions where perceptual training can be difficult, e.g. in stroke rehabilitation.

Disclosures: **A. Marzoll:** None. **E. Keßel:** None. **H.R. Dinse:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.16/BB26

Topic: F.01. Human Cognition and Behavior

Support: BMBF Grant 01GQ1003B

Title: Contingency awareness as a prerequisite for contextual fear conditioning

Authors: ***P. MEYER**, C. BAEUCHL, M. HOPPSTAEDTER, H. FLOR;
CIMH, Heidelberg Univ., Mannheim, Germany

Abstract: We employed functional magnetic resonance imaging to investigate the acquisition of contextual fear in humans in in a sample of 96 young healthy subjects using two context-pictures, of which one (CS+) was paired with an aversive electrical shock (US) in 50 percent of the trials, whereas the other picture (CS-) was not shock associated. The context-pictures were comprised of identical sets of features in different spatial arrangements and demand a configural context processing strategy in order for successful conditioning to occur. In addition, we simultaneously recorded skin conductance responses (SCR) and asked subjects to rate the stimuli on arousal, valence and contingency awareness. On the single subject level, contrast estimates that reflect the effect of fear conditioning (CS+unpaired > CS-) were calculated. Then we created two groups of subjects named “contingency aware” (n = 41) and “contingency unaware” (n = 55) based on their differential rating of the perceived contingency between CS+ and US. The comparison of blood oxygen level dependent (BOLD) activation between both groups (contingency aware > contingency unaware) revealed responses in bilateral insula, inferior frontal gyrus, superior medial gyrus and bilateral inferior parietal lobule. In addition, there was significant transient activation of the hippocampus in contingency aware subjects but not in contingency unaware ones. SCRs of the whole experiment were divided into three time bins and

CS+unpaired trials were compared to CS- trials using separate t-tests. In all three time bins, SCRs were larger for CS+unpaired than for CS- in contingency aware subjects compared to contingency unaware subjects. Finally, the stimulus ratings revealed significant differences between the groups with higher ratings on arousal and emotional valence for CS+ than for CS- in contingency aware relative to contingency unaware subjects. Taken together, our results support the notion that consciousness awareness of the CS+/US contingency is necessary for the acquisition of contextual fear when the discrimination of contexts is particularly demanding.

Disclosures: **P. Meyer:** None. **C. Baeuchl:** None. **M. Hoppstaedter:** None. **H. Flor:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.17/BB27

Topic: F.01. Human Cognition and Behavior

Support: SFB grant 874

Title: Survival of the fittest gamer or: How video games improve probabilistic learning

Authors: ***S. SCHENK**, R. K. LECH, B. SUCHAN;
Neuropsychology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: The present fMRI study examines the effects of video game playing on the strategy usage and the related neural correlates of probabilistic learning. Previous studies have shown that successful probabilistic learning correlates positively with activity in the tail of the caudate nucleus, the putamen and the extrastriate cortex (middle temporal gyrus). While the putamen is associated with the analysis of stimulus-outcome probabilities, the caudate nucleus integrates performance and represents cognitive control demands. Besides, both implicit and explicit strategies could be used in probabilistic classification learning. Sixteen healthy right-handed video-gamers and fifteen healthy right-handed non-gamers were scanned in a 3T scanner while performing a modified version of the weather prediction task, a well-known probabilistic classification learning task. The behavioral data yield evidence for a better categorization performance of gamers, particularly at more uncertain card combinations. Furthermore, a post-experimental questionnaire showed that video-gamers used mostly a multi cue strategy while non gamers used more often an explicit single cue or singleton strategy. Analysis of the functional imaging data revealed a between group effect. Non-gamers showed higher activations of the left middle temporal gyrus as well as in the superior parietal lobe. It seems that probabilistic categorizations required more effort for non-gamers under the usage of an explicit top down driven visual processing. Separate group analyses yielded in both groups activations in the left tail of the caudate and the right putamen. However, while gamers showed higher activations in

the left tail of caudate for high probable weather choices, non-gamers showed higher activations in the right putamen. Gamers exhibit strong performance integration and high cognitive control demands, whereas non-gamers place emphasis on stimulus-outcome probabilities. Overall, video-gamers presented a more implicit processing. These results are in line with previous studies which suggested that video game playing enhances probabilistic inference, which promote cognitive flexibility and improves performance in a wide variety of tasks.

Disclosures: **S. Schenk:** None. **R.K. Lech:** None. **B. Suchan:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.18/BB28

Topic: F.01. Human Cognition and Behavior

Title: Successive training of two tasks in three-week periods still leads to behavioral interference

Authors: ***M. SENDEN**, G. LANGE, A. RADERMACHER, R. GOEBEL, P. DE WEERD; Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands

Abstract: Previous studies [1,2] have shown that skill learning, although quite stable in general, can be interfered with by additional training at a later time on similar tasks/stimuli. Commonly, these behavioral interference effects are attributed to the disruption of a time-limited offline memory consolidation process. Interestingly, behavioral interference has been shown to occur for time intervals of up to 24 hours [1] or longer [2], which raises the question whether it is best accounted for by disrupted consolidation. Alternatively, memory traces after their formation may remain malleable, and behavioral interference might occur when memory formation in two tasks recruits a largely shared (i.e. insufficiently segregated) neuronal population subjected to incompatible requirements on network connectivity. To evaluate this possibility, we first implemented a learning mechanism in a recurrent model of orientation selectivity characterized by representational overlap. We used this neural network model to simulate the effects of orientation discrimination learning as well as of varying contributions of consolidation on the interference between two tasks. In these tasks, efficient orientation discrimination learning required opposite network connectivity changes. Secondly, we designed an empirical experiment, in which training between the two tasks was done serially in time blocks of three weeks, thereby maximizing the possibility that one task would induce a fully consolidated memory trace before onset of the next one. Nevertheless, we found strong interference between the two tasks, similar to what has been observed for intervals in the range of 0-24h [1]. Our model simulations show that our results can be more straightforwardly explained in terms of reactivation and representational competition than in terms of disruption of consolidation. [1] Been, M., Jans, B., & De Weerd, P. (2011). Time-limited consolidation and task interference: no

direct link. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(42), 14944–51; [2] Caithness, G., Osu, R., Bays, P., Chase, H., Klassen, J., Kawato, M., Wolpert, D.M., Flanagan, J. R. (2004). Failure to consolidate the consolidation theory of learning for sensorimotor adaptation tasks. *The Journal of Neuroscience, 24*(40), 8662-71. Supported by: NWO VICI grant to P.D.W. (#453-04-002). Authors M.S. and R.G. were supported by an ERC grant (agreement no. 269853). Authors G.L. and M.S. contributed equally.

Disclosures: M. Senden: None. **G. Lange:** None. **A. Radermacher:** None. **R. Goebel:** None. **P. De Weerd:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.19/BB29

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01 EY021755

The John Templeton Foundation

Title: Examining changes in functional connectivity during human perceptual learning with population receptive fields

Authors: *V. R. BEJJANKI, N. B. TURK-BROWNE;
Dept. of Psychology and Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: When we repeatedly look at the same stimulus, we get better at seeing it. How does such perceptual learning occur in the brain? A recent computational theory (Bejjanki et al., 2011, *Nat Neurosci*) proposed that changes in connectivity between visual areas may be responsible, by increasing the fidelity with which task-relevant information is transmitted. In the current study, we sought to test this proposal in humans using fMRI. We tested the hypothesis that perceptual learning at one location in space should lead to increased connectivity between voxels in areas V1 and V4 that have population receptive fields (pRFs) with coverage of the trained location. Moreover, the extent to which a particular pair of V1 and V4 voxels increase in connectivity should be related to how much their pRFs overlap with each other. We used a task in which observers detected one of two novel shape stimuli, each a mirror reflection of the other, with one shape presented in the upper-right quadrant and the other in the upper-left quadrant. Observers were trained to detect one of the shapes (the “trained” shape), with sensitivity for this shape and the other “control” shape being assessed before and after training. fMRI data were collected during training over six runs. In a second session, we retinotopically mapped the most effective visual field location for voxels in areas V1-V4 and combined this information with published estimates of pRF extents in these areas (Harvey & Dumoulin, 2011, *J Neuro*) to derive a pRF for

each voxel. Observers showed improved behavioral sensitivity after training, with greater improvement for the exposed vs. control shape. With the fMRI data, we inferred “background” connectivity between voxels in areas V1 and V4 by regressing out stimulus-evoked responses and global noise sources, and computing correlations in the residual BOLD timeseries. We then computed a weighted average of the background connectivity between each V4 voxel and the V1 voxels in its pRF, where the weights were determined by the distance between pRF center locations according to a Gaussian function. Consistent with our hypothesis, there was greater background connectivity between voxels in areas V1 and V4 whose pRFs overlapped the trained vs. control locations across the six training runs. Additional analyses examine pRFs at a finer spatial scale for the envelope and contour of the trained shape. These findings support the proposal that perceptual learning alters functional networks in the visual cortex to improve the transmission of learned information.

Disclosures: V.R. Bejjanki: None. **N.B. Turk-Browne:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.20/BB30

Topic: F.01. Human Cognition and Behavior

Support: Italian Ministry of Health (RF-2009-1543811)

Title: Subjective symptoms in MRI operators

Authors: *S. ZAFFINA, JR, V. CAMISA, A. SANTORO, M. VINCI, A. ANTICO, V. CANNATÀ, P. DERRICO;
Children's Hosp. bambino Gesù, Rome, Italy

Abstract: Introduction Operators working in proximity to a MRI scanner are exposed to high levels of electromagnetic fields (EMF) generated by these devices. In these MRI operators an increase of some symptoms, as vertigo, headache, nausea, metallic taste has been reported. This problem is relevant, especially considering that the number of operators, and the strength of MRI scanners is increasing. We have studied the prevalence of symptoms in a group of MRI operators and in controls. Methods In a group of 82 MRI operators (52 M, 30 F, mean age 40 ± 9.5), working in 2 different hospitals, and in 31 Controls (18M, 13F mean age 40 ± 9.9) the occurrence of subjective symptoms has been collected by a physician using a questionnaire based on data reported in previous studies. Main factors inducing symptoms, as ear diseases or drugs, were excluded; MRI operators and controls were similar for all the relevant characteristics except for exposure in MRI. Results We compared the mean number of total symptoms and frequent symptoms in exposed and not exposed and both the mean and the distribution

(respectively 3.8 ± 2.4 SD vs 3.7 ± 2.7 SD and 1.09 ± 1.4 SD vs 1.07 ± 1.5 SD) were similar. Considering total symptoms vertigo was statistically significant comparing cases and controls (Chi square 5,84 p <0,01) while as regards the frequent symptoms in male the difference is significant for headache (Chi square 7,1 p <0,007). Discussion These data confirm a higher prevalence of symptoms in people exposed to MRI possibly due to the EMF; the prevalence of individual symptoms are largely comparable with those finded in the recent studies (tiredness 20%, sleep disorders 13%, nausea and metallic taste 2,2%). An unexpected observation never described before is the different prevalence of symptoms between the two sexes. The collection of data is ongoing, further questionnaire from other subjects will be gathered. This work was supported by grant from the Italian Ministry of Health (RF-2009-1543811)

Disclosures: **S. Zaffina:** None. **V. Camisa:** None. **A. Santoro:** None. **M. Vinci:** None. **A. Antico:** None. **V. Cannatà:** None. **P. Derrico:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.21/BB31

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Senior Research Fellowship: 100227

Title: Aberrant precision in autism: behaviour, computational modelling and noradrenergic function

Authors: ***R. P. LAWSON**[1], C. MATHYS[1], G. REES[2,1];
[1]Wellcome Trust Ctr. for Neuroimaging, [2]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom

Abstract: New theories of brain function in Autism Spectrum Disorder (ASD) propose that an imbalance of the precision (or gain) ascribed to sensory inputs, relative to top-down predictions of those inputs, might underlie the perceptual atypicalities in the disorder (Lawson et al, 2014, Front. Hum. Neuro., 8, 302). Precision itself rests on the optimal representation of environmental uncertainty and the action of neuromodulators, such as noradrenaline. Here we employ computational models of behaviour and indices of central noradrenergic function to explore aberrant precision setting in ASD. Adults with ASD (n=21) and age and ability-matched neurotypical controls (NT; n=18) took part in a probabilistic associative learning task. Participants heard either a high or low tone, followed by a briefly presented image, and were required to make a button press response indicating whether the image was a face or a house. The probabilistic associations between the tone and the images changed over time. Concurrent pupillometry provided trial-by-trial biomarker of central noradrenergic function (Samuels et al.,

2008, Curr Neuropharmacology, 6, 1-19). Data were modelled using the Hierarchical Gaussian Filter (Mathys et al., 2014, Front. Hum. Neuro., 8, 825), allowing the quantification of individual learning under different levels of uncertainty. NT adults showed a slowing of reaction and an increase in error rates in response to images of increasing outcome unexpectedness (OU), whereas ASD adults showed no modulation of error rates and a significantly reduced RT modulation as a function of OU. Strikingly, the amount of RT slowing in response to images of high OU correlated with autistic traits across all individuals. NT adults also showed a trend towards the predicted pupil dilation to increasing OU whereas ASD adults did not. Modelling of the RT data to characterise individual learning suggests that group status can be predicted from model parameters that characterise the influence of 'high-level' environmental uncertainty on RT. These data provide evidence that adults with ASD are less able than NT adults to use the probabilistic structure in the environment to guide behaviour. Such a pattern of responses is consistent with reduced sensitivity to environmental uncertainty, which itself determines the precision (gain) that one should ascribe to sensory inputs, relative to top-down predictions of those inputs. An absence of pupillometric response to OU further suggests aberrant neural precision (central noradrenergic function) in ASD.

Disclosures: R.P. Lawson: None. **C. Mathys:** None. **G. Rees:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.22/BB32

Topic: F.01. Human Cognition and Behavior

Title: The amount of practice and the acquisition of the internal model in visuomotor learning

Authors: *C. YAMADA[1], Y. ITAGUCHI[3,2], K. FUKUZAWA[1];
[1]Waseda Univ., Tokyo-To, Japan; [2]Waseda Univ., Tokyo, Japan; [3]Sapporo Med. Univ., Sapporo, Japan

Abstract: Our goal is to reveal the relationship between the amount of practice and a visuomotor learning. To investigate the time course of practice-dependent error reduction, we used a tracking task, which less likely to allow a strategic control than a reaching task. In the tracking task, participants were required to follow a randomly moving target as accurate as possible during a trial. Participants were assigned to one of four experimental groups, and completed the tracking task in two successive days. On Day 1, they performed 20 Normal only sessions, 20 Rotation included sessions, 10 Normal only sessions, or 10 Rotation included sessions (N20, R20, N10, and R10). On Day 2, all of the participants completed 10 Rotation included sessions. Additional participants were recruited to define the baseline performance, and they performed 20 Normal only sessions on Day 1 and 10 Normal only sessions on Day 2. We calculated the distance

between the target and the mouse cursor to define the tracking error. We compared two error measures to characterize the performance among the four conditions: the error on the last rotation trial (Last Error), and the increase of the tracking error between the end of Rotation trial and the subsequent Normal trial (After-Effect). The results showed that there was no difference in the Last Error between Day 1 (R10 vs R20) and Day 2 (among the four conditions). The participants in the four groups decreased tracking error to the same level on Day 2 regardless of the different amount of rotation practice on Day 1. In addition, participants only who completed R20 sessions showed a positive After-Effect in the end of Day 1 and 2. These results suggested that the amount of practice which can lead to the learning plateau is not enough to form an internal model for the novel visuomotor environment. Further, the occurrence of positive after-effects might reflect that the visuomotor adaptation is completed, which lasts at least to the next day. The current results support the idea that an extra practice after the learning plateau is needed for better learning.

Disclosures: **C. Yamada:** None. **Y. Itaguchi:** None. **K. Fukuzawa:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.23/BB33

Topic: F.01. Human Cognition and Behavior

Support: BBSRC

ESRC

NCMH

NISCHR

Title: Dissociable roles for the inferior longitudinal fasciculus and the fornix in perception for faces and scenes

Authors: ***M. A. POSTANS**[1], B. COAD[1], M. ALY[2], C. J. HODGETTS[1], D. E. J. LINDEN[1], A. D. LAWRENCE[1], K. S. GRAHAM[1];
[1]Psychology, Cardiff Univ., Cardiff, United Kingdom; [2]Princeton Neurosci. Inst., Princeton, NJ

Abstract: Inter-individual variation in structural properties of the inferior longitudinal fasciculus (ILF) and the fornix has been shown to be predictive of discrimination accuracy for pairs of visually similar faces and scenes, respectively (Postans et al., 2014). A limitation of this previous work, however, was the use of small sets of repeated stimuli, because over time, stimulus learning and memory may have contributed to performance measures. It is therefore unclear whether category-sensitive contributions of the ILF and fornix to discrimination

accuracy, extend to perception for trial-unique stimuli. In addition, the previous work treated perception as a unitary construct, but recent investigations indicate that two functionally independent types of perception contribute to visual discrimination performance. The relative contributions of these two components - termed *state*- and *strength-based* perception - can be separated using receiver operating characteristics (ROCs; Aly et al., 2012; 2013). Here, we asked whether diffusion-MRI indices of ILF and fornix microstructure (fractional anisotropy, FA, and mean diffusivity, MD), would correlate with markers of state- and/or strength-based perceptual discrimination of trial-unique faces and scenes, respectively. Diffusion-weighted MR images were obtained from healthy participants who had completed a task in which they made confidence-based same/different discriminations to trial-unique, visually similar face and scene pairs. Participants' confidence-based responses were used to plot ROCs, from which state- and strength-based perception were estimated for each stimulus category. Mean FA and MD values were computed for the fornix and ILF of each participant, which were reconstructed using deterministic white-matter tractography. ILF MD was found to correlate with markers of perception for faces but not scenes, whereas fornix MD was found to correlate with markers of perception for scenes but not faces. These findings are consistent with the category-sensitive white matter-behaviour relationships reported in Postans et al., (2014) and show that the dissociable roles for the ILF and fornix in perception for faces and scenes extends to perceptual discriminations that minimize the contribution of learning and memory to performance. This research was supported by funds awarded by the Biotechnology and Biological Sciences Research Council (BBSRC), the Economic and Social Research Council (ESRC), the National Centre for Mental Health (NCMH), and the National Institute for Social Care and Health Research (NISCHR).

Disclosures: **M.A. Postans:** None. **B. Coad:** None. **M. Aly:** None. **C.J. Hodgetts:** None. **D.E.J. Linden:** None. **A.D. Lawrence:** None. **K.S. Graham:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.24/BB34

Topic: F.01. Human Cognition and Behavior

Support: NIMH Intramural Research Program

Title: Repetition priming in object naming is associated with repetition suppression, earlier termination of activity, and changes in task-engaged neural synchrony

Authors: *S. J. GOTTS, A. OSSOWSKI, S. C. MILLEVILLE, A. MARTIN;
Lab. of Brain and Cognition, NIMH/NIH, Bethesda, MD

Abstract: Object repetition commonly leads to long-lasting improvements in the speed and accuracy of identification ("repetition priming"), along with decreased neural activity ("repetition suppression"). In the current study, we use fMRI and overt picture naming (N=32 subjects) to evaluate several prominent models of the relationship between repetition priming and suppression. Subjects named a set of 100 pictured objects 3 times prior to fMRI. During fMRI, they overtly named "old" pictures randomly intermixed with "new" pictures that were matched in category and name frequency. The use of a slow-event-related fMRI design aided in movement artifact separation, as well as improved isolation of BOLD responses to individual trials for the purposes of task-based functional connectivity analyses. In addition to the standard effects of repetition suppression in occipitotemporal and lateral frontal regions, we also observed a significant alteration in the time course of the BOLD response following repetition, with earlier termination for old pictures, consistent with predictions of Facilitation and Predictive Coding models. Functional connectivity analyses performed on the individual item responses further revealed that: 1) increased connectivity with left inferior frontal cortex during old relative to new pictures predicted greater priming magnitudes across subjects (consistent with the Synchrony model), and 2) increased connectivity with dorsal parietal regions during new pictures also predicted greater priming through slower new RTs, consistent with slowed responding due to a heightened novelty/orienting response. Thus, priming appears to reflect a combination of more rapid, synchronized activity to old pictures and novelty-related slowing and heightened attention to new pictures.

Disclosures: **S.J. Gotts:** None. **A. Ossowski:** None. **S.C. Milleville:** None. **A. Martin:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.25/BB35

Topic: F.01. Human Cognition and Behavior

Support: James S. McDonnell Foundation 624748

NIA RO1-AG034613

Title: Virtual environmental enrichment through video games

Authors: *G. D. CLEMENSON, C. E. STARK;
UC Irvine, Irvine, CA

Abstract: In animals, it is well understood that exposing animals to a more stimulating environment, known as environmental enrichment, can stimulate neuroplasticity and improve hippocampal function and performance on hippocampally-mediated memory tasks. We are interested in whether these manipulations that successfully enhance cognition (or mitigate

cognitive decline) also influence humans in a similar manner. Although humans, in many ways, already live in an enriched environment, we are constantly adapting to new experiences and situations within our own environment on a daily basis. Here, we hypothesize that the exploration of the vast and visually stimulating virtual environments within video games, is a human correlate of environmental enrichment. We show that video gamers who specifically favor 3D video games performed better on a demanding recognition memory task that assesses participants' ability to discriminate highly similar lure items from repeated items to behaviorally tax hippocampal pattern separation. In addition, after two weeks of training on the 3D video game Super Mario 3D World, naïve video gamers showed improved mnemonic discrimination ability and improvements on another hippocampal-dependent task - a virtual water maze. Two control conditions (passive and training in a 2D game, Angry Birds), showed no such improvements. In addition, we provide preliminary data exploring the functional involvement of the human hippocampus during video game play. Thus, our results suggest that modern day video games may provide meaningful stimulation to the human hippocampus.

Disclosures: G.D. Clemenson: None. **C.E. Stark:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.26/BB36

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 26870426

Title: Fast knowledge-mediated visual disambiguation process in humans: a magnetoencephalographic study

Authors: *T. URAKAWA[1,2], K. OGATA[2], T. KIMURA[2], Y. KUME[2], S. TOBIMATSU[2]; [1]Tokyo Univ. of Sci., Dept. of Applied Physics, Katsushika-ku, Japan; [2]Dept. of Clin. Neurophysiol., Kyushu Univ., Fukuoka, Japan

Abstract: Ever-changing visual environment is full of noisy visual scenes. To adequately adapt to such environment, the disambiguation of the noisy scenes with prior knowledge is essential and needs to be implemented in the brain as fast as possible under the limited capacity of visual image processing. However, the temporal profile of the disambiguation process has not yet been fully elucidated. The present study attempted to determine how quickly knowledge-mediated disambiguation began to proceed along visual areas after the onset of a two-tone ambiguous image using magnetoencephalography. From the predictive coding framework, we focused on activity reduction for the two-tone ambiguous image as an index of the implementation of disambiguation. Twelve healthy volunteers participated in this study. An unambiguous

photograph was first repetitively presented, and three different two-tone images were then successively presented in random order. Participants were first required to look at a photograph and then to report whether or not the following two-tone images were disambiguated with the prior photograph on a trial-by-trial basis. Under this scheme of image presentation, two sessions were established. The two-tone images used in one session were identical with those in the other session. The disambiguation was set to be difficult in the first session and to be easy in the second session for one of the two-tone images with a replacement of a preceding photograph, keeping the other ambiguous images difficult in disambiguating for both sessions. Source analysis revealed activity reduction at around 120 ms in the lateral occipital area but not for the preceding activity (about 115 ms) in the cuneus when participants disambiguated the two-tone image. Further analyses showed that the activity reduction was not observed for the other two-tone images which were not perceptually disambiguated. These results suggested that knowledge-mediated disambiguation may be implemented as early as approximately 120 ms following an ambiguous visual scene, at least in the lateral occipital area, and provided an insight into the temporal profile of the disambiguation process of a noisy visual scene with prior knowledge.

Disclosures: **T. Urakawa:** None. **K. Ogata:** None. **T. Kimura:** None. **Y. Kume:** None. **S. Tobimatsu:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.27/BB37

Topic: F.01. Human Cognition and Behavior

Support: Karp Discovery Award

Title: The human retrosplenial cortex and thalamus code head direction in a global reference frame

Authors: ***J. SHINE**[1], J. P. VALDÉS-HERRERA[1], M. HEGARTY[2], T. WOLBERS[1,3]; [1]DZNE, Magdeburg, Germany; [2]Dept. of Psychological and Brain Sci., Univ. of California, Santa Barbara, CA; [3]Ctr. for Behavioral Brain Sci., Magdeburg, Germany

Abstract: Spatial navigation is a multisensory process involving integration of visual and body-based cues. In rodents, head direction (HD) cells, which are most abundant in the thalamus, integrate these cues to code facing direction. Human fMRI studies examining HD coding in virtual environments have reported effects in retrosplenial complex and (pre-)subiculum, but not the thalamus. Furthermore, HD coding appeared insensitive to global landmarks. These tasks, however, provided only visual cues for orientation, and attending to global landmarks did not

benefit task performance. In the present study, participants explored a VE comprising four separate locales, surrounded by four global landmarks. To provide body-based cues, participants wore a head-mounted display so that physical rotations changed facing direction in the VE. During subsequent MRI scanning, subjects saw stationary views of the environment and judged whether their orientation was the same as in the preceding trial. Parameter estimates extracted from anatomical masks of the retrosplenial cortex and the thalamus revealed significantly reduced BOLD response in both regions associated with repeating HD. Moreover, consistent with rodent findings, the signal did not continue to adapt over repetitions of the same HD. These results were supported by a whole-brain analysis showing additional repetition suppression in the precuneus. Together, our findings demonstrate that (i) consistent with the rodent literature, the thalamus integrates body-based, and visual, orientation cues, (ii) global reference frame cues can be used to integrate HD across separate individual locales, and (iii) immersive training procedures providing full body based cues may help to elucidate the neural mechanisms supporting spatial navigation.

Disclosures: **J. Shine:** None. **J.P. Valdés-Herrera:** None. **M. Hegarty:** None. **T. Wolbers:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.28/BB38

Topic: F.01. Human Cognition and Behavior

Support: Air Force Office of Scientific Research Grant FA9550-10-1-0385 to R.P

Title: Distraction suppression and video game training: far transfer effects to fluid intelligence

Authors: ***A. E. HARWOOD**[1,2], D. CISLER[2], R. PARASURAMAN[2], P. GREENWOOD[2]; [2]Psychology, [1]George Mason Univ., Fairfax, VA

Abstract: There is ongoing interest in developing interventions to heighten fluid intelligence (Gf). Based on evidence that (a) action "first person shooter" video games (AVGs) improve distraction suppression (Bavelier et al., 2012) and (b) high Gf depends on ability to ignore distracting events (Wiley et al., 2011), we hypothesized that a training regimen which increased distraction suppression should also induce far transfer to Gf. We tested this by comparing two different training task regimens aimed at distraction suppression (AVG and visual perception) with training task aimed at reasoning (active control). Specifically, 10 hours of the following training was randomly assigned: (a) first person shooter AVG, (b) commercial visual perception training (previously found to transfer to Gf, Berry et al., 2010), (c) real time strategy game Rise of Nations. We assessed changed performance on a battery of transfer tasks administered before

and after training. Visual perception training transferred positively to distractor suppression and episodic memory while the AVG had a negative impact on both. Reasoning training had little effect. In contrast to previous claims about AVG, we did not find transfer to Gf. These results confirm our previous finding that perception training is particularly effective in transferring to Gf (Strenziok et al., 2014).

Disclosures: A.E. Harwood: None. **D. Cisler:** None. **R. Parasuraman:** None. **P. Greenwood:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.29/BB39

Topic: F.01. Human Cognition and Behavior

Title: Differential contributions of positive and negative reinforcement in temporal order visual processing

Authors: *K. HU[1], A. K. ANDERSON[2], W.-M. LUH[4], E. D. ROSA[3];
[1]Cornell University, Ithaca, NY, Ithaca, NY; [2]Dept. of Human Develop., Cornell Univ., Human Neuroscience Institute, NY; [3]Dept. of Human Develop., Cornell Univ., Cornell University, NY; [4]Cornell MRI Facility, Cornell University, NY

Abstract: Previous research has made significant progress in identifying the neural basis of the reward effect in cognitive and perception. However, the neural mechanism that positive and negative reinforcement shapes low level visual information remains poorly understood. Here we investigated the interaction between value reinforcement across spatial frequency channels. Positive and negative reinforcement was used in two experiments, respectively. In the conditioning phase, value was manipulated by linking fast and accurate responses to horizontal or vertical gratings of varying spatial frequency. In study 1, participants received positive reinforcement (gains) for correct responses. In study 2, participants received negative reinforcement (avoiding losses) for correct responses. Following conditioning phases, participants were required to observe those conditioned stimuli while conducting side-based temporal order judgment (TOJ) task where stimuli were presented at varying stimulus onset asychronies (SOA). We found that bilateral activation along the calcarine fissure in primary visual cortex was sensitive to SOA temporal order. Positive reinforcement resulted in similar pattern of visual cortical recruitment relative to neutral trials, further recruiting the cuneus and right posterior cingulate cortex (CGp). By contrast, Negative reinforcement (avoiding losses) did not have such an effect on primary visual cortical regions responsive to SOA. These results suggest positive but not reinforcement influences the neural bases of onset detection in primary

visual cortex and that CGp and cuneus may play special roles for early visual perception in positive reinforcement.

Disclosures: **K. Hu:** None. **A.K. Anderson:** None. **W. Luh:** None. **E.D. Rosa:** None.

Poster

351. Perceptual and Spatial Learning

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 351.30/BB40

Topic: F.01. Human Cognition and Behavior

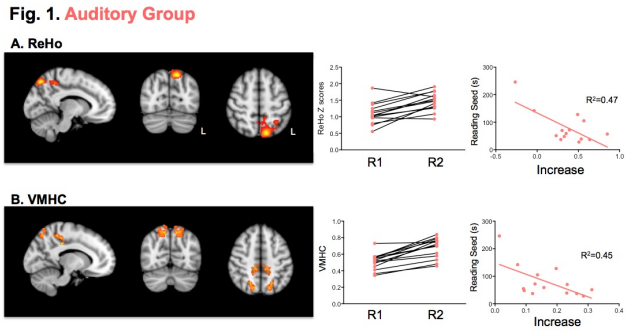
Support: NSF grant #SMA-1041755

Title: Resting-state fMRI correlates of rapid brain plasticity following brief auditory exposure

Authors: ***M. S. KOYAMA**[1], S. ORTIZ-MANTILLA[2], L. HELFERSTAY[2], J. PARASCANDO[2], C. ROESLER[2], J. MORGANBYRNE[2], M. P. MILHAM[1], A. A. BENASICH[2];
[1]Child Mind Inst., New York, NY; [2]Rutgers Univ., Newark, NJ

Abstract: INTRODUCTION: Neuroimaging studies have investigated long-term experience-induced brain plasticity, particularly in the auditory domain (e.g., musicians). However, only a few fMRI studies have examined rapid changes following sensory experience, which most focused on the motor domain. Here, we used resting-state fMRI (R-fMRI) to address this gap, mapping neural correlates of rapid plasticity following auditory experience in adults. METHODS: 24 healthy monolingual adults (mean age/SD=25/15yrs), with no reported history of learning or psychiatric disorders, participated in behavioral (WASI full IQ; WRAT word reading) and R-fMRI experiments. 14 participants were passively exposed in the scanner to complex tones, whereas 10 controls experienced only a silent gap. Each 5 min period was inserted between two R-fMRI scans. The auditory stimuli used were novel to all participants, and the groups did not differ in age, gender, IQ, reading, or head motion. R-fMRI was acquired for whole-brain 180 volumes (TR=2ms; voxel-size=3x3x3; duration=6m). MRI data were preprocessed by CPAC. We focused on two R-fMRI derivatives, Regional Homogeneity (ReHo) and Voxel-Mirrored Homotopic Connectivity (VMHC), which are considered to assess regional specialization and inter-hemispheric functional connectivity, respectively. At the group level, a paired t-test was performed to map effects of the auditory exposure. RESULTS: For ReHo (Fig 1.A), the auditory group showed a significant increase in the left precuneus (Pre)/superior parietal lobule (SPL), which was associated with shorter response time for word reading. That is, the greater the increase, the faster the reading. The same brain-behavior relationship was observed for VMHC in Pre/SPL (Fig 1.B). These results suggest that brain's ability to alter intrinsic functional properties in the parietal cortex, immediately after new sensory experience,

may index higher cognitive efficiency in adults. We will further discuss the findings in light of functions of the default mode and attention networks.



Disclosures: **M.S. Koyama:** None. **S. Ortiz-Mantilla:** None. **L. Helferstay:** None. **J. Parascando:** None. **C. Roesler:** None. **J. Morganbyrne:** None. **M.P. Milham:** None. **A.A. Benasich:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.01/BB41

Topic: F.01. Human Cognition and Behavior

Support: UK MoD project funding

Title: The utility of reinforcement learning for EEG based passive auditory-neurofeedback

Authors: ***M. DINOV**, A. GUNASEKARA, R. LEECH;
Computational, Cognitive and Clin. Neuroimaging Lab., Imperial Col. London, London, United Kingdom

Abstract: Electroencephalography (EEG) based neurofeedback (NFB) is a biofeedback approach that uses a person's brain signals for performance optimization in healthy and in clinical groups. There are techniques that automate the process of influencing cognitive states, which are more convenient as they require no effort on the part of the subject - these are termed passive NFB. One passive NFB method of temporarily improving reaction time (RT) during sustained attention tasks in both clinical and healthy groups is phasic alerting. Though phasic alerting is not new, we extend previous work by a) optimizing various alerting-related parameters previously unexplored to this extent and b) creating an extensible and general framework for EEG based attentional monitoring and improvement system that is primarily based on reinforcement learning (RL), but also uses EEG signal prediction using artificial neural networks (ANNs) for predicting attentional lapses before they happen and phase-dependent

alerting. We used Q-Learning for RL and show here that RL is a flexible and powerful approach for exploring a EEG-defined brain state space for various parameters. The RL-based approach successfully learns to prefer alerting in higher alpha states (lower attention states) and not disturb the user during lower alpha power states (higher attention states), with no explicit programming to do so. This is achieved through two simple reinforcement rules. We have implemented these methods in the context of the Psychomotor Vigilance Task (PVT), which is a commonly used vigilance task. We compared and found differences between four variants of the task: simple adaptive PVT with alerting (saPVTa), RL PVT with alerting (rlPVTa), PVT where alerting occurs without reference to the real time alpha power (PVTa), alerting only within the inter-stimulus interval (ISI) for 20 percent of ISIs, and PVT with no alerting as a reference. RL allows an ongoing realtime optimization of various NFB-related parameters without the need to specify a threshold a priori as in the saPVTa. The RL also allows for easy integration of non-alpha EEG signals, and we have explored some of these uses and show that non-alpha attention-related signals can successfully be found and used, by themselves or in conjunction with the alpha signals. However, additional brain signals quickly enlarge the state space. Sufficient exploration of the RL state space can take a long time for larger state spaces, so simpler or shorter tasks may benefit from using a more constrained (but still functional) alpha-only state space (to the exclusion of other brain signals) or even a simple moving threshold approach.

Disclosures: **M. Dinov:** None. **A. Gunasekara:** None. **R. Leech:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.02/BB42

Topic: F.01. Human Cognition and Behavior

Support: NIMH grant F32 MH102009-01A1

Title: Nobody expects the Spanish Inquisition: Expectations about the source of surprise dictate the relationship between feedback-related EEG signals and learning

Authors: ***M. R. NASSAR**[1], R. BRUCKNER[2], M. J. FRANK[1];
[1]Metcalf Hall, Brown Univ., Providence, RI; [2]cDepartment of Educ. and Psychology, Freie Univ. Berlin, Berlin, Germany

Abstract: Successful decision-making requires learning expectations based on experienced outcomes. This learning should be calibrated according to the surprise associated with an outcome, as well as the most likely source of surprise. For example, when occasional change points are expected, surprising outcomes can render past information irrelevant and demand increased learning. In contrast, when signal corruption is expected to occur occasionally,

surprising outcomes can suggest a corrupt signal that should be ignored by learning systems. Previous work has identified feedback-related EEG deflections sensitive to surprise and may play a role in behavioral updating, but it is unknown whether these signals, or behavior, are sensitive to the source of surprise. Here we explore whether, and how, humans combine surprise with source expectations to rationally govern learning. To do so we collected EEG and behavioral data using a computerized task that required subjects to estimate the target of a virtual cannon based on noisy observations. On each trial the subject observed the circular position of a single cannonball and adjusted the position of a "shield" in order to block the next one. The exact dynamics of the cannon were the key task manipulation: in "change point" blocks the cannon remained stable but underwent occasional discontinuous changes, whereas in "signal corruption" blocks cannon aim drifted from trial-to-trial but cannonball positions were occasionally drawn uniformly from an independent process. Subjects adjusted learning in accordance with surprise and their expectations about the source thereof. In change point blocks participants increased learning when confronted with surprising cannonball positions, whereas in the signal corruption blocks participants decreased learning in response to surprising outcomes. Thus the effects of surprise on learning depended critically on subjective expectations about the source of surprising observations. Despite this behavioral interaction, feedback-related EEG signals were agnostic to the source of surprise. A large positive medial prefrontal deflection peaking 350 ms after outcome presentation was enhanced for surprising outcomes in both conditions equally. Computational fits to behavior showed that the impact of this signal on learning differed across conditions: larger EEG deflections predicted more learning in change point blocks but less learning in signal corruption blocks. Taken together these findings suggest that medial prefrontal surprise signals do not naively reflect increased behavioral updating, but may be used adaptively to modulate learning in either direction.

Disclosures: **M.R. Nassar:** None. **R. Bruckner:** None. **M.J. Frank:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.03/BB43

Topic: F.01. Human Cognition and Behavior

Support: Cedars-Sinai Startup Funding

Conte Center Fuding

Title: A human single-neuron correlate of error monitoring in anterior cingulate cortex and pre-supplementary motor area neurons

Authors: *Z. FU[1,4], A. N. MAMELAK[4], I. B. ROSS[6], J. M. CHUNG[4], R. ADOLPHS[2], U. RUTISHAUSER[3,5];
[1]Div. of Engin. and Applied Sci., [2]Div. of the Humanities and Social Sci., [3]Div. of Biol., Caltech, Pasadena, CA; [4]Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA; [5]Neurosurg., Cedars-Sinai Med. Ctr., Pasadena, CA; [6]Huntington Mem. Hosp., Pasadena, CA

Abstract: To monitor our own actions, detect and compensate for errors is a key feature of human executive function and learning. Given the conceptual importance of error feedback in human and machine learning, significant effort has been devoted to search for such a mechanism, and substantial progress has been made over the last 20 years with the discovery of an electroencephalographic correlate recorded from the scalp: the error-related negativity (ERN). However, how this ERN might be reflected in the activity of single neurons remains unknown. We used human intracranial electroencephalography (iEEG) and single unit recordings to investigated error detection in anterior cingulate cortex (ACC) and pre-supplementary motor area (pre-SMA). 24 epilepsy surgery candidates with implanted intracranial depth electrodes participated in the study and performed 71 sessions of a speeded version of a color-word Stroop task. Behaviorally, subjects showed a robust Stroop interference effect (155 ms) and post-error slowing effect (76ms). We isolated 232 neurons in ACC and 187 in SMA/pre-SMA. A substantial subset of these well-isolated neurons in ACC and pre-SMA changed firing rate immediately after subjects made an error, well before feedback was presented. 24% (55/232) of ACC and 22% (41/187) of pre-SMA neurons increased firing rate significantly while 15% (34/232) of ACC and 23.5% (44/187) of pre-SMA neurons decreased their firing rate significantly after errors relative to correct trials. A significant percentage of ACC, but not SMA, neurons was modulated in the correct trials that followed an error, a phenomena termed post-error modulation (13.4% increase while 12% decrease firing rate). This suggests that the ACC is involved in cognitive control mechanisms that detect and compensate for errors. We simultaneously recorded iEEG from macro electrodes and local field potential from microwires. In both we identified error-related potentials in ACC and pre-SMA. These error-related potentials had a negative and positive going component with waveform and latency similar to classical studies of the ERN (Falkenstein et al 1990, Gehring et al,1993). Theta power increased significantly more in error trials than correct trials (ACC: 44% of all sessions, SMA: 60% of all sessions). The temporal patterns of the ERN and error-related neuronal activity suggest that we have identified a single-neuron correlate of the classic scalp-recorded ERN. In surprising contrast, we did not find any single-neuron correlates of conflict monitoring. Together, our findings provide the first potential circuit-level mechanism for well-known theories of error monitoring by ACC and pre-SMA.

Disclosures: Z. Fu: None. **A.N. Mamelak:** None. **I.B. Ross:** None. **J.M. Chung:** None. **R. Adolphs:** None. **U. Rutishauser:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.04/BB44

Topic: F.01. Human Cognition and Behavior

Support: ERC Advanced Grant (HUMVOL)

Title: Agency, learning and reward: a study using temporal binding

Authors: S. DI COSTA[1], H. THÉRO[2], *P. HAGGARD[1];
[1]Univ. Col. London, London, United Kingdom; [2]École normale supérieure, Paris, French Guiana

Abstract: The sense of agency refers to the feeling that we control our actions and, through them, effects in the outside world. Laboratory research on sense of agency often lacks ecological validity. Studies typically investigate associations between one simple action, and an arbitrary, direct effect of the action. Outside the laboratory, actions are embedded in a rich perceptuomotor, affective and social landscape. People frequently select one action from several possible in a given situation, based on a goal they wish to achieve. Thus, the sense of agency seems intimately related to value-based decision making. However, few studies have attempted to link sense of agency with outcome valence. Accordingly, we combined measures of agency (described below) with reward-based decision-making, seemingly for the first time. We used a reversal learning approach, which requires participants to track occasional changes in the mappings between two keypresses and two outcomes, and adjust actions accordingly, to maximise reward. In three experiments, participants chose between two action alternatives, which differed in reward probability. We also measured shifts in perceived time of actions and subsequent outcomes, or "temporal binding" as an implicit proxy for sense of agency. In the first experiment, switching between actions produced greater binding of the action towards the outcome than repeating actions. Further, negative outcomes showed stronger binding of outcome towards action than positive outcomes. Both effects were absent when participants made timing judgements before the reward value was known, suggesting a reconstructive effect of rewards on action experience (Experiment 2). Experiment 3 disentangled the effects of reward and contingency, with an additional condition having the same occurrence patterns as unrewarded trials, but irrelevant to reward and to overall performance. Switching between actions again produced stronger action binding, but no effect of valence on outcome binding was found. This suggests that valence effects on sense of agency may be consequences of the frequent and contingent relation between action and positive outcomes in instrumental learning. Finally, we used mixed-model regressions to explore the sources of variability in individual timing judgements across all 3 experiments. This showed a single reliable relation at the trial-to-trial level, between strong action binding and failure to earn reward on the previous trial. Our work suggests a novel relation between sense of agency and reward-based decision-making.

Disclosures: **S. Di Costa:** None. **H. Théro:** None. **P. Haggard:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.05/BB45

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant K99/R00 MH094438

Title: Positive reinforcement enhances encoding of upcoming information

Authors: *D. G. DILLON[1], M. FRANK[2], D. BADRE[2], D. A. PIZZAGALLI[1];
[1]Ctr. for Depression, Anxiety and Stress Res., McLean Hospital/Harvard Med. Sch., Belmont, MA; [2]Cognitive, Linguistic & Psychological Sci., Brown Univ., Providence, RI

Abstract: Each day we form many short-term memories, most of which rapidly decay. However, some episodes stay with us for years, even decades. What triggers memory retention? We hypothesized that positive reinforcement is critical: by activating brain reward networks that communicate with medial temporal lobe memory regions, positive reinforcement may promote consolidation. To test this hypothesis in Experiment 1, 36 adults viewed 240 natural and man-made images and decided to keep or reject each one. Decisions were reinforced by delivery of "rewards" (monetary feedback) or "zeros" (no monetary feedback), and the reinforcement rate for stimulus/response pairings varied over the two blocks of the task. Participants returned a day later for a surprise recognition memory test. We predicted that recognition accuracy (hit rate) would be higher for images followed by rewards versus zeros, but this was not the case for images from either encoding block (effect of current feedback on hit rate, by block: $Zs < 1.40$, $ps > 0.16$). However, there was a reliable effect of prior feedback: recognition accuracy was higher for images preceded by delivery of rewards versus zeros (effect of prior feedback on hit rate, block 1: $Z = 4.42$, $p < 0.001$; block 2: $Z = 3.03$, $p = 0.002$). In Experiment 2, 22 adults completed the same task during functional magnetic resonance imaging. Replicating Experiment 1, recognition accuracy was higher for images preceded by rewards versus zeros (block 1: $Z = 4.28$, $p < 0.001$; block 2: $Z = 1.95$, $p = 0.051$), but was unaffected by feedback delivered following image presentation ($Zs < 1$). A whole-brain "reward versus zero" contrast revealed activation of bilateral hippocampus (left, $Z = 4.59$; right, $Z = 5.09$) and ventromedial prefrontal cortex ($Z = 4.91$), regions consistently implicated in episodic encoding and reward valuation (cluster $ps < 0.05$, FWE-corrected). Because the memory tests were unexpected, these results cannot be attributed to strategic effects at encoding. Thus, positive reinforcement elicits robust hippocampal activation and appears to facilitate incidental encoding of upcoming information in this task, leading to better retention. To strengthen this interpretation, ongoing analyses are focused on determining whether there is a significant interaction between the hippocampal response to (1) reward versus zero delivery and (2) subsequently remembered versus forgotten

images. These findings may be relevant to understanding the co-occurrence of anhedonia and memory deficits in certain psychiatric conditions, such as unipolar depression.

Disclosures: **D.G. Dillon:** None. **M. Frank:** None. **D. Badre:** None. **D.A. Pizzagalli:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.06/BB46

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1460604

NIMH RO1 MH080066-01

NIMH F32 MH102009-01A1

Title: Dissociable effects of reinforcement valence and learning rate on incidental encoding and consolidation of episodic memory

Authors: ***A. I. JANG**[1], M. NASSAR[1], D. DILLON[2], M. FRANK[1];
[1]Brown Univ., Providence, RI; [2]McLean Hospital/Harvard Med. Sch., Belmont, MA

Abstract: Maintaining accurate beliefs in a changing environment requires learning from rewards and penalties, but also adjusting the rate of learning according to environmental statistics. For example, learning should be enhanced when outcomes are surprising or beliefs are uncertain but reduced when beliefs are reliable and outcomes are noisy. In such scenarios, adaptive learning is thought to involve two major neuromodulators: dopamine, which is thought to signal outcome valence relative to expectations, and norepinephrine, which is thought to signal how much should be learned from a new outcome. These two neuromodulators are also thought to affect memory encoding and consolidation through actions on the temporal lobe. However, recent studies designed to elicit dopamine release during decision-making have reported very different effects on subsequent memory recall for trial-specific stimuli. Here we try to clarify the effects of dopamine in a behavioral task by de-correlating outcome valence (thought to be signaled by dopamine) from overall learning rate (thought to be signaled by norepinephrine) and measuring the effects of both factors on incidental memory encoding and consolidation. To do so, we designed a novel 2-part task that required reinforcement learning amid change points followed by a surprise recognition memory test that was administered online via Amazon Mechanical Turk. In part 1, inferring the best choice ('play' for potential reward, or 'pass') on each trial required subjects to integrate information about trial value (sampled independently on each trial and displayed numerically), reward probability (evolving according to a change-point structure and learned by integrating feedback across trials), and stimulus

category (depicted by a unique exemplar image). Subjects rationally adjusted learning from trial-to-trial and these learning rates were largely uncorrelated with expected and actual trial values. The recognition memory test was administered either 5 minutes or 24 hours after the decision task and required subjects to report whether each of a series of images had been used as a category exemplar during the decision task. We found that feedback valence and learning rate made dissociable contributions to subsequent recall. Positive feedback on play trials elicited improvements in consolidation for the subsequent trial exemplar, whereas surprise-driven learning enhancements led to diminished encoding of the previously displayed exemplar. This work should help clarify seemingly contradictory accounts of the role of feedback signals in episodic memory consolidation and informs future studies directed at inferring mechanisms.

Disclosures: **A.I. Jang:** None. **M. Nassar:** None. **D. Dillon:** None. **M. Frank:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.07/BB47

Topic: F.01. Human Cognition and Behavior

Support: RO1HD053793

Rothschild Fellowship

Title: Reinforcement mechanisms underlie use-dependent plasticity in human motor behaviors

Authors: ***F. MAWASE**[1], S. UEHARA[1], A. BASTIAN[2,4], P. CELNIK[1,2,3];
[1]Physical Med. and Rehabil., [2]Neurosci., [3]Neurol., Johns Hopkins Univ., Baltimore, MD;
[4]Kennedy Krieger Inst., Baltimore, MD

Abstract: Use dependent plasticity (UDP) has been defined by the presence of movement direction biases resulting from movement repetition. Although these biases have been observed in many transcranial magnetic stimulation (TMS) and behavioral human studies, little is known about the mechanisms that underlie UDP. In particular, the effects of reinforcement mechanisms in previous studies are hidden behind the goal of the task and likely go unnoticed. Therefore, one suggested hypothesis is that UDP is insensitive to reward and reflects the history of the repeated actions. Alternatively, it is conceivable that reinforcement mechanisms may also play a role in UDP. Here we assessed UDP in a series of brain stimulation and behavioral experiments in which reinforced repeated actions and non-reinforced repeated actions could be distinguished. We performed two complementary experiments with independent samples. The first experiment explored the effect of implicit reinforcement during skill learning on UDP, while the second experiment explored the effect of explicit reinforcement during simple repetition on UDP. In the first experiment, two groups of subjects learned a pinch force task where forces mapped to lateral

cursor displacement. Subjects were asked to move the cursor sequentially to set of five targets by applying isometric forces in one predefined direction. Group 1 trained on consistent trials of a logarithmic map, which allows performance improvement, whereas group 2 were exposed to random maps that markedly decreased the number of successful trials and thus prevented accumulation of learning. A second experiment included two additional groups of subjects that were instructed to move the cursor to a single target by applying isometric force. We dissociate reinforced repeated actions from non-reinforced repeated actions by giving explicit binary reward associated with task success (group 3) and random rewards independent to task success (group 4), respectively. We found that TMS-evoked movement directions were biased toward directions that were either implicitly or explicitly reinforced (group 1 and 3), suggesting that rewarded actions might be a mechanism that is critical to develop movement direction biases. Critically, these biases were not present when repetitions were not associated with learning (group 2) or when they were accompanied by random reinforcement (group 4). Altogether, our findings support a crucial role of reinforcement mechanisms on use-dependent plasticity and suggest that use-dependent processes might underlie formation of motor habits, in our case expressed as biases towards previous rewarded actions.

Disclosures: **F. Mawase:** None. **S. Uehara:** None. **A. Bastian:** None. **P. Celnik:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.08/BB48

Topic: F.01. Human Cognition and Behavior

Title: Behavioral studies with the cue-approach task show it can enhance preferences towards faces and fractals

Authors: *T. SALOMON[1], R. BOTVINIK[2], S. ISRAEL[2], T. SCHONBERG[3];
[2]Sagol Sch. of Neurosci., [3]Dept. of Neurobiology, The George S. Wise Fac. of Life Sci. and Sagol Sch. of Neurosci, [1]Tel Aviv Univ., Tel Aviv-Yafo, Israel

Abstract: How the brain constructs value is a basic question in decision neuroscience. We recently presented replicable results with of cue-approach task, where the mere-association of a tone and a button press led to preference changes of snack food items without external reinforcement or context changes. Here we present novel findings showing that the task can be used to enhance preferences of a wide range of stimuli such as faces and fractals. Two independent samples were collected: 28 participants (16 F, ages 18-32, (mean 24.8 ± 3.8)) completed the task with neutral and happy face images. Another sample with 24 participants (14 F, ages 18-27, (23.2 ± 2.2)) completed the task with color fractal images. To obtain individual rankings of faces and fractals, we used a binary ranking method. We then split the items and

defined half of the items as higher valued (HV) and the other items as lower valued (LV) items. Images of 60 faces or fractals were presented on the screen for 1 sec. Participants were instructed to press a button as fast as they could only when they heard a tone. Out of the 60 items, 20 or 24 (10 or 12 HV and 10 or 12 LV) were consistently associated with the tone in the face and the fractal task, respectively. The items were presented 12 times each during training. In the probe phase, participants were presented with pairs of items from the same value category that had similar rankings, but only one of the items in each pair was associated with the signal in the training phase (e.g. GoHV vs. NoGoHV). Participants were asked to choose one item per trial based on their preferences. To measure the success of training we compared the ratio of choices of the Go vs. NoGo items (within HV or LV pairs). We found that faces associated with the go-signal during training were chosen significantly more often during probe: for HV items 58% of trials p=0.003; LV items in 58% of trials, p=0.03. We found that fractals associated with the go-signal during training were chosen significantly more often during probe: for HV items 64% of trials p=0.002; LV items in 61% of trials, p=0.03. Thus, we show that cue-approach training can be extended to items that have no pre-existing preference and experience such as unfamiliar faces and fractals. The ability to change preference towards faces suggests a novel therapeutic approach for depression to enhance liking of happy stimuli. The known neural mechanism of face processing will allow us to track the underlying neural mechanisms of the preference change in the task. The ability to enhance choices of fractals suggests that preference towards neutral and positive stimuli can be enhanced with the cue-approach paradigm, which speaks to the generality of the underlying mechanism.

Disclosures: **T. Salomon:** None. **R. Botvinik:** None. **S. Israel:** None. **T. Schonberg:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.09/BB49

Topic: F.01. Human Cognition and Behavior

Title: Modulating EEG source activities in the frontal cortex by using tEIC increases learning rate during decision making

Authors: ***M. WATANABE**[1,2,3], A. HOTTA[1], A. MATANI[1];
[1]Grad. Sch. of Information Sci. and Technol., [2]Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan; [3]RIKEN Brain Sci. Inst., Wako, Japan

Abstract: During value-based decision making, action values which are learned by trial and error can be altered by unexpected environment changes. Facilitating learning rates in response to environmental changes should be effective to adapt such changes. Studies have shown that neural activities in the frontal cortex reflect environmental changes and suggested that this region

may have a role of modulating learning rate (Behrens et al., Nature Neuroscience, 2007; McGuire et al., Neuron, 2014). However, it has not yet been investigated whether activation in the frontal cortex is sufficient to increase learning rate. We examined a role of the frontal cortex in value learning by facilitating or inhibiting neural activities with transcranial extracellular impedance control (tEIC), which is a noninvasive technique to increase (with Type I tEIC) or decrease (with Type II tEIC) EEG source activities by controlling extracellular impedance (Matani et al., PLoS ONE, 2014). A pilot study revealed that environmental changes were clearly observed in EEG between Fz and the right electrodes. We hence attached tEIC between these two locations. Six volunteer subjects participated in the following decision making task. Subjects selected left or right target on a PC monitor to earn a reward in each trial as high as possible. The two alternatives were set to provide rewards following normal distributions with each different mean and the common variance, which enables subjects to learn which selection leads better outcome. When learning seemed to be over, or selection showed on-side dominance, the environment, or the reward setting, was changed unexpectedly to them. Half of environmental changes were randomly selected and tEIC was applied for first 10 trials after the selected changes; the rest half was set to Sham accordingly. This study received approval from the Ethics Committee of the University of Tokyo. We demonstrated that Type I tEIC significantly increased learning rates compared to Sham (Wilcoxon signed-rank test, $p=0.03$), while it did not affect other parameters such as exploiting rate ($p>0.05$). Inhibiting the neural activities with Type II tEIC resulted in no significant changes in learning rate or other parameters. These results show that neural responses observed in the frontal EEG are quit relevant to learning rate. Moreover, since Type I tEIC, at least in theoretical, increases neural inputs in the cortex, the frontal cortex may integrate information on environmental changes with other neural inputs and provide output to facilitate learning.

Disclosures: **M. Watanabe:** None. **A. Hotta:** None. **A. Matani:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.10/BB50

Topic: F.01. Human Cognition and Behavior

Support: ARC-SRI: Science of Learning Research Centre (project number SR120300015)

Title: Neural networks underlying aversive context conditioning in humans

Authors: ***L. MARSTALLER**, H. BURIANOVÁ, D. REUTENS;
Univ. of Queensland, Brisbane, Australia

Abstract: Previous studies have shown that the hippocampus plays an important role in the contextual modulation of conditioned fear responses. However, the functional connectivity between limbic and cortical regions underlying aversive context conditioning in humans is still unclear. The purpose of this fMRI study was to investigate brain connectivity during aversive context conditioning in healthy male adults. The participants were presented with two simple visual stimuli in two alternating, visually distinct contexts. In the danger context but not the safe context, one of the stimuli was paired with cutaneous electro-tactile stimulation (CS+, 60% reinforcement), whereas the other, control stimulus (CS-) was never paired with stimulation. Electrophysiological data showed a significant difference in skin conductance responses between CS+ and CS- in the danger context as well as between CS+ in the danger and the safe context. Analysis of functional connectivity with bilateral amygdala revealed an extensive fear acquisition network that was engaged during CS+ in both contexts as well as during CS- in the danger context. The fear acquisition network included bilateral amygdala, hippocampus, caudate, and cingulate, somatosensory, middle frontal, and anterior temporal cortices. The results further showed a right lateralized temporal-limbic-occipital network that was activated whenever a stimulus was presented in the safe context. Importantly, this network was anti-correlated with activity in the striatum, the salience, and fronto-parietal networks. Our results suggest that the acquisition and contextual regulation of fear in humans can be differentiated by the functional connectivity of the right amygdala with its left hemispheric counterpart and the hippocampus.

Disclosures: **L. Marstaller:** None. **H. Burianová:** None. **D. Reutens:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.11/BB51

Topic: F.01. Human Cognition and Behavior

Title: Up and down: Punishment, relief and emotional instability

Authors: *E. ELDAR[1,2], P. DAYAN[3], R. J. DOLAN[1,2];
[1]Wellcome Trust Ctr. for Neuroimaging, UCL, London, United Kingdom; [2]Max Planck Univ. Col. London Ctr. for Computat. Psychiatry and Ageing Res., London, United Kingdom; [3]Gatsby Computat. Neurosci. Unit, Univ. Col. London, London, United Kingdom

Abstract: Challenges to maintaining emotional equanimity come from both dashed and delivered hopes of the arrival of rewards and avoidance of punishments. We tested 48 participants whose emotional instability had been assessed through a questionnaire on two gamble-based card games involving probabilistic increases and decreases in both aversive and appetitive outcomes. In one game, a gamble could result in either avoiding or receiving a painful electrical shock; in the other, winning or losing a monetary reward. Participants could always

decline a gamble and opt for an intermediate outcome (i.e., 50% probability of shock or no change in monetary bonus). In both games, participants played with three different decks of cards, and had to learn by trial and error how likely a gamble was to be successful with each. We assessed responsivity to the different types of outcomes by examining how participants adjusted their gambles given the outcomes they had observed for each deck, and also monitored their galvanic skin responses. Selectivity to emotional instability was only apparent in the game involving shocks. Whereas most participants mainly learned through repeating successful gambles that had helped them avoid shocks, the more unstable participants primarily learned by declining previously unsuccessful gambles for which they had received shocks ($r = 0.66$, $p = 0.002$ corrected). In addition, high emotional instability scores were associated with weaker skin conductance responses to avoided-shock outcomes. Our findings suggest that a weaker response to a decrease in aversive outcomes might play a role in emotional instability.

Disclosures: **E. Eldar:** None. **P. Dayan:** None. **R.J. Dolan:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.12/BB52

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust 093270/Z/10/Z

Niels Stensen stipendium

Title: Adaptive prediction error coding in the human midbrain and striatum correlates with behavioral adaptation and learning efficiency

Authors: *K. M. DIEDEREN, T. SPENCER, P. FLETCHER, W. SCHULTZ;
Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Learning to accurately predict upcoming rewards presents a challenge of everyday life. Most rewards are not singular events with constant magnitude, but fluctuate from one moment to the next, thus complicating learning. Learning may, however, be improved by scaling updates to match environmental variability. I.e., prediction errors (PEs) are more meaningful when rewards vary less. Importantly, the mechanism by which the PE signal is normalized to fluctuations in reward value may be facilitated by neurons encoding these errors in units of standard deviation (SD). Such adaptive coding puts PEs of different ranges on the same scale and enables the brain, despite its limited processing capacity, to discriminate between PEs equally well regardless of their absolute magnitudes. Although previous fMRI studies reported adaptive PE coding in contexts where task contingencies are explicit, it is, unclear whether the human brain encodes PEs normalized to reward variability during learning. Here, we investigated

adaptive coding during learning and established whether the degree of adaptive coding correlates with behavioral adaptation and task performance. Participants predicted the magnitude of upcoming rewards drawn from distributions with different SD's (5, 10 and 15). After each prediction, participants received a reward, yielding trial by trial PEs that spanned the full range of distributions. Fitting with computational models showed that participants decreased learning rates in conditions with a higher SD, suggesting behavioral adaptation. In line with the notion of adaptive coding, regression slopes in the midbrain and ventral striatum were steeper for PEs occurring in distributions with smaller SDs. Indeed, BOLD responses varied with normalized (PE/SD) instead of non-normalized PEs. Adaptation was not instantaneous but only occurred as trials progressed. In addition, BOLD encoding slopes for conditions with medium variability (SD 10) decreased when paired with a lower SD (SD 5) and increased when paired with a higher SD (SD 15), revealing additional contextual effects on adaptation. Adaptive PE coding was paralleled by behavioral adaptation to reward variability, revealing a tight relation between behavioral and neural measures of adaptation. Indeed, the individual degree of behavioral adaptation correlated strongly with the individual extent of adaptive PE coding in the striatum and midbrain. Crucially, a higher degree of BOLD adaptation in the midbrain and ventral striatum was related to improved task performance. These results suggest that adaptive PE coding during learning facilitates behavioral adaptation and supports efficient learning.

Disclosures: K.M. Diederen: None. **T. Spencer:** None. **P. Fletcher:** None. **W. Schultz:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.13/BB53

Topic: F.01. Human Cognition and Behavior

Title: Spontaneous eyeblink rate predicts increased recruitment of a model-based learning strategy

Authors: *T. C. SHI, L. E. HUNTER, Y. L. SOUFIAN, J. H. DECKER, C. A. HARTLEY; Sackler Inst. for Developmental Psychobiology, Weill Cornell Med. Col., New York, NY

Abstract: Individuals can recruit different strategies to make everyday decisions. Reinforcement learning theories distinguish two such strategies: a "model-based" strategy builds and recruits a cognitive model of the decision state space to flexibly evaluate and select actions, while a "model-free" strategy simply increments the probability of repeating previously successful actions. Deficits in model-based learning, proposed to support prospective goal-directed decisions, are thought to heighten vulnerability to multiple disorders of compulsivity (Voon et al., 2015). However, determinants of individual variability in the recruitment of these two strategies are only beginning to be understood. Previous studies using pharmacological

manipulations (Wunderlich et al., 2012) and PET imaging (Deserno et al., 2015) have demonstrated a link between increased central dopamine levels and increased model-based choice. Based on an extensive literature associating increased central dopamine levels with higher spontaneous eyeblink rate (SEBR), we hypothesized that individuals exhibiting higher resting SEBR would show more model-based decision-making. To test this hypothesis, we recorded resting SEBR in healthy adults who subsequently completed a two-stage sequential reinforcement learning task designed to dissociate model-free and model-based choice behavior (Daw et al., 2011). We fit a hybrid reinforcement learning model to quantify the degree to which participants' choice behavior reflected model-based computations. Preliminary results revealed a significant positive correlation between resting SEBR and model-based choice (indexed by a continuous weighting parameter). Corroborating previous evidence that dopamine modulates the recruitment of model-based versus model-free learning, these findings further suggest that resting SEBR represents a valid measure for examining the relationship between dopaminergic function and choice behavior.

Disclosures: T.C. Shi: None. **L.E. Hunter:** None. **Y.L. Soufian:** None. **J.H. Decker:** None. **C.A. Hartley:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.14/BB54

Topic: F.01. Human Cognition and Behavior

Support: BMBF Germany, FKZ 01EO1001

DFG Germany, SFB 1052 Obesity mechanisms

Title: Cardiac concomitants of decreased reinforcement learning in individuals with obesity

Authors: J. KUBE[1,2], D. MATHAR[1,2], L. KASTNER[1,2], A. HORSTMANN[1,2], A. VILLRINGER[1,2,3,4], *J. NEUMANN[1,2];
[1]MPI For Human Cognitive and Brain Sci., Leipzig, Germany; [2]IFB Adiposity Diseases, Univ. Med. Ctr., Leipzig, Germany; [3]Clin. of Cognitive Neurology, Univ. Hosp., Leipzig, Germany; [4]Mind & Brain Institute, Berlin Sch. of Mind and Brain, Humboldt-University, Berlin, Germany

Abstract: Objective: Positive and negative feedback provides substantial information about our environment and drives learning and behavioral adaptation. Phasic variations in cardiac responses have been found to be sensitive to motivationally significant stimuli and are typically associated with a strong and partly prolonged deceleration for task-relevant feedback [1,2]. Previously, it has been reported that individuals with obesity show altered choice behavior and neural responses in feedback-based food and non-food related tasks [3,4,5]. Here, we

investigated the cardiac concomitants of positive and negative feedback in monetary gain and loss processing and their association to reinforcement-based learning in individuals with obesity. Methods: Obese (BMI >=30 kg/m2) and lean (BMI 18.5-24.9 kg/m2) adults comparable in age and education performed a probabilistic learning task. In separate gain, loss, and neutral monetary conditions participants learned to choose between a high and low reinforcement probability option in order to maximize their financial outcome. An electrocardiogram was acquired during this task. Participants' feedback-evoked heart period (HP) was analyzed using three inter-beat-intervals (IBI) around the time of feedback onset. Additionally, we modelled participants' learning performance using a Q-learning algorithm. Results: Behavioral analysis and computational modelling revealed decreased learning performance for participants with obesity in both monetary gain and loss trials. The presentation of negative feedback was associated with a typical prolonged HP deceleration that was particularly pronounced in the early stage of the experiment. Individuals with obesity showed a blunted cardiac deceleration compared to lean controls, which was related to learning performance. Conclusion: Our results suggest that obesity is associated with decreased feedback-based learning performance. Altered physiological responses to non-food reinforcement stimuli could be an indicator of an altered feedback-based monitoring system impairing the reinforcement-based learning process in individuals with obesity. References: [1] Crone et al., J Exp Child Psychol, 2006, 95:99-116. [2] Crone et al., Biol Psychol, 2003, 64:143-56. [3] Balodis et al., Biol. Psychiatry, 2013, 73:877-86, 2013 [4] Horstmann et al., Front Hum Neurosci, 2011, 10:5:58 [5] Stice et al., J Abnorm Psychol, 2008, 117:924-35

Disclosures: **J. Kube:** None. **D. Mathar:** None. **L. Kastner:** None. **A. Horstmann:** None. **A. Villringer:** None. **J. Neumann:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.15/BB55

Topic: F.01. Human Cognition and Behavior

Support: Guggenheim Fellowship

Office of Naval Research Grant N00014-15-1-0033

Title: Neural oscillatory patterns during associative learning

Authors: *B. M. ROBERTS, A. CLARKE, C. RANGANATH;
Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Much of our learning in everyday situations depends on our ability to learn new and sometimes arbitrary associations. This kind of learning has been studied in rodents and

nonhuman primates, and is known to depend on interactions between distributed brain regions including the prefrontal cortex, hippocampus, and striatum. In rodents, theta oscillations have been correlated with associative learning, but it is unclear whether these findings generalize to human associative learning. The purpose of this study was to characterize changes in oscillatory activity during learning of arbitrary visuomotor associations. We recorded EEG from 18 subjects recruited from the UC Davis student community during performance of an associative learning task. Each trial began with presentation of an abstract shape at the center of the screen. After a short delay period, subjects were prompted to press a button on the keyboard (numbered 1, 2, 3, or 4). Following each response, feedback was provided to indicate whether the response was correct or incorrect. Subjects were instructed to use the feedback to help learn which button presses corresponded to each of the shapes. For each block (4 blocks total), subjects learned button presses for 12 distinct abstract shapes. Each shape was presented 12 times in randomized order. Behavioral results indicated that subjects learned the majority of these associations, and at the final repetition, subjects performed at an average of 87.04± 2.63% accuracy, which was significantly above chance ($p=5.8x10^{-123}$). Preliminary analyses of EEG data revealed differences in cue and delay period oscillatory activity across repetitions. Specifically, frontal theta power increased across learning, but become maximal once performance reached around 80% correct, and subsequently decreased during the final repetitions. These results indicate the importance of frontal theta oscillations during learning and strengthening of associations, but suggest that theta activity declines once the associations are well-learned. Learning-related changes in oscillatory activity also occurred during the delay period, particularly within alpha and gamma bands. Unlike theta oscillations, alpha and gamma power increased throughout learning. These oscillations may be more related to the maintenance of the visual stimulus during preparation for the motor response. Further analyses will investigate feedback-related activity and relate oscillatory power changes to behavioral performance. The present results, nonetheless, suggest that associative learning is associated with robust changes in brain activity that might be used as biomarkers in clinical studies.

Disclosures: **B.M. Roberts:** None. **A. Clarke:** None. **C. Ranganath:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.16/BB56

Topic: F.01. Human Cognition and Behavior

Support: Government of the Russian Federation

Title: The effect of economic competition on the neural mechanisms of decision-making

Authors: *M. MARTINEZ-SAITO[1], B. GUTKIN[1,2], A. SHESTAKOVA[1], V. KLUCHAREV[1]; [1]Ctr. for Cognition and Decision Making, Higher Sch. of Econ., Moscow, Russian Federation; [2]Ecole Normale Superieure, Paris, France

Abstract: Behavioral economics has extensively studied how people make economic decisions in environments with different levels of supply and demand (i.e. with different levels of economic completion). However, the neural mechanisms underpinning such decisions remain unidentified. Here we study the neural mechanisms underlying decisions in different conditions of economic competition. Additionally, we aim to investigate the learning processes that lead to adaptive bargaining strategies, and how these are modulated by the degree of economic competition. Fifteen subjects played the role of buyers in simultaneous games against different numbers of prerecorded buyers and sellers. We used a modified Ultimatum Game (double auctions) in 50-minute 3T fMRI scanning sessions. Overall, the game allowed us to identify the effects of competition (number of sellers and buyers) on subjects' willingness to pay (the size of bids). Behavioral results demonstrated that subjects adjusted their trading price during the game based on the perceived competitiveness of the environment. We observed a progressive, yet incomplete convergence towards the optimal strategy predicted by a game-theoretic analysis. Intriguingly, the data hint at two separable learning processes involved: the subjects' overall scales of bid values are mainly influenced by the market environment, whereas subjects' trial-by-trial adjustment of bid values instead display a skewed distribution modulated by the outcome of the previous trial. Preliminary fMRI data analysis showed significant differential activations and differential dynamics of the activity in the basal ganglia in the different competitive conditions. The results of the pilot study indicate that people learn to alter trading price based on the perceived competitiveness of the environment and suggest a profound role of the dopaminergic system in behavioral adaptations during economic competition.

Disclosures: M. Martinez-Saito: None. **B. Gutkin:** None. **A. Shestakova:** None. **V. Klucharev:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.17/BB57

Topic: F.01. Human Cognition and Behavior

Support: PDF Pilot Award

CIHR Fellowship

Title: Effects of dopamine on the consolidation of incremental learning: implications for Parkinson's disease

Authors: *M. SHARP[1], K. FOERDE[2], K. DUNCAN[1], D. SHOHAMY[1];
[1]Psychology, Columbia Univ., New York, NY; [2]Psychology, New York Univ., New York, NY

Abstract: There is accumulating evidence that dopamine plays an important role in various types of learning and memory. Much of this work has focused on the acquisition stage of learning, in particular the role of striatal dopamine in stamping in stimulus-response associations. Much less is known about the factors that influence the consolidation of these associations. Some clues come from the study of explicit memory where there is evidence that through its facilitation of long-term potentiation, the presence of dopamine at the time of encoding is critical for the consolidation of long-lasting episodic memories. Additionally, dopamine is thought to contribute to the specificity of long-term memory by selectively enhancing rewarding or otherwise motivationally salient memories. Here we aim to address two questions: First, does dopamine at the time of acquisition similarly lead to enhanced consolidation of incrementally learned stimulus-response associations? Second, does dopamine differentially enhance the consolidation of incremental learning from positive versus negative outcomes? We adapted an incremental learning paradigm that allows us to test the persistence of learning over time. Participants gradually learn probabilistic associations and on each trial, the outcome is indicated by a trial-unique image that, by its category assignment, signals whether they were correct or incorrect. The key feature of our design is that we test participants on two sessions separated by a one-week delay. This allows us to assess off-line consolidation of learning by comparing performance immediately after acquisition to performance after a long delay. Our design also allows us to test the effect of reward on off-line consolidation and, in parallel, to test the effect of reward on the consolidation of episodic memory for the trial-unique images. Preliminary data in healthy control participants shows that participants learn successfully, that both learning from positive feedback and learning from negative feedback persist similarly over the one-week delay, and that episodic memory is better for reward-associated images. To directly test the role of dopamine in off-line consolidation we will also present data from patients with early stage Parkinson's disease. One group of patients is tested ON medications at both sessions and one group is OFF medications at both sessions, thus allowing us to isolate the effects of dopamine on off-line consolidation from its effects on acquisition and performance.

Disclosures: **M. Sharp:** None. **K. Foerde:** None. **K. Duncan:** None. **D. Shohamy:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.18/BB58

Topic: F.01. Human Cognition and Behavior

Support: Natural Sciences and Engineering Research Council (NSERC) CREATE IRTG 449313-2014

Title: Decision-making in Parkinson's patients during a strategic game

Authors: *A. C. PARR, B. C. COE, G. PARI, D. P. MUNOZ;
Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: During social games, each player's actions and their associated outcomes change dynamically based on their opponent's actions, and experience is used to predict future outcomes by estimating the value of actions. In reinforcement learning models, a reward prediction error signal drives learning the value of actions, widely thought to be encoded by midbrain dopaminergic neurons. Deficits in reward-based learning, and corresponding decreases in striatal prediction error signalling, have been observed in Parkinson's disease (PD), which is associated with the loss of dopaminergic input to the striatum. Dopaminergic medication results in increased learning rates in dynamic decision-making tasks requiring reinforcement learning in PD. We investigated stochastic decision-making in patients with mild PD (stage 1-2) and age-matched controls while they competed in a color-based version of the strategic game, Matching Pennies, against a dynamic computer opponent that exploited biases in player's response patterns. Players selected one of two different colored visual targets, and were rewarded if their selection matched that of the opponent. Results were contrasted with a control decision-making task wherein reward was linked to a specific visual stimulus, but was otherwise similar to Matching Pennies in terms of sensory input, motor output, and reward rate. Participants indicated their choices with either a saccade or a button press. Both groups completed 2 sessions; PD patients both off- and on-medication. The PD group displayed a leftward direction bias during both tasks, which correlated with the severity of motor deficits on the contralateral side. During Matching Pennies, the PD group displayed a color bias, but no significant differences in overall reward rate compared to controls. PD patients had higher error rates during the control task, resulting in significantly lower reward rates compared to controls. In the PD group, we observed a significant main effect of medication on reward, and an interaction of medication by task, with a trend toward higher reward rates in the off-medication condition than the on-medication condition during Matching Pennies, however, this effect was not observed during the control task. We demonstrate that differences in dynamic choice behavior in PD can be investigated using strategic games.

Disclosures: **A.C. Parr:** None. **B.C. Coe:** None. **G. Pari:** None. **D.P. Munoz:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.19/BB59

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 5R03DA031583-02

Title: Is dopamine necessary for reward-related incidental learning improvements? Evidence from patients with Parkinson's disease

Authors: *M. V. FREEDBERG[1], J. SCHACHERER[2], K.-H. CHEN[3], K. NARYANAN[3], E. UC[3], E. HAZELTINE[2];
[2]Psychology, [3]Neurol., [1]The Univ. of Iowa, Iowa City, IA

Abstract: Midbrain dopamine neurons respond to both the presence of an unexpected reward and the absence of an expected reward (Schultz, 1998). This dopamine reward-prediction signal has been inferred to be involved in various forms of learning including incentive learning and instrumental learning (Wachter et al., 2009; Frank et al., 2004). However, recently it has been demonstrated that rewards can be used to bolster incidental learning, even when participants demonstrate little to no awareness of which associations were rewarded (Freedberg et al., in review). The primary pathology in Parkinson's disease (PD) is the degeneration of dopaminergic neurons in the midbrain with projections to brain regions important for motor function, cognition, and behavior. Here, we examine the role of dopamine neurotransmission in supporting reward-related improvements by comparing the performance and learning of patients with Parkinson's disease (PD), who are not demented and live independently, to age-matched comparisons. Participants performed a single-session experiment in which they were asked to respond to pairs of faces in which half the pairs were linked to a monetary reward. Immediately following training of the rewarded and unrewarded combinations participants performed a transfer block where they were asked to perform the same pairs without rewards. The data indicate that patients with PD showed significantly less reward-related incidental learning improvements compared to age-matched comparisons ($F(1, 10) = 9.794$, $p < 0.05$). These results may emphasize a critical role of dopamine neurotransmission in acquiring rewarded information as well as highlight the potential learning deficits of patients with PD in acquiring rewarded information.

Disclosures: **M.V. Freedberg:** None. **J. Schacherer:** None. **K. Chen:** None. **K. Naryanan:** None. **E. Uc:** None. **E. Hazeltine:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.20/BB60

Topic: F.01. Human Cognition and Behavior

Support: Economic and Social Researhc Council (ES/L012995/1)

Title: Perceptual learning affects post-sensory processing on a visual decision making task

Authors: *J. DIAZ, M. PHILIASTIDES;
The Inst. of Neurosci. and Psychology (INP), Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Training and experience lead to long-lasting improvements in our ability to make decisions based on ambiguous sensory information - a phenomenon commonly referred to as perceptual learning. Despite the obvious prevalence of perceptual learning in everyday life, its underlying neural substrates are still not well understood. Recent primate electrophysiology experiments have provided compelling evidence that perceptual learning during decision making does not change how sensory information is represented in the brain, but rather how sensory representations are interpreted, particularly by higher-level areas involved in the decision process itself (Law & Gold, 2008). To test this interpretation formally in humans we recorded electroencephalography (EEG) during a visual two-alternative forced choice discrimination task (face vs. car discrimination). Using this task we previously identified two temporally distinct EEG components discriminating between the two image categories, which were associated with early encoding of the sensory evidence and post-sensory encoding of the decision evidence, respectively (Philiastides and Sajda, 2006). Here we measured how the strength of these components (i.e. our ability to discriminate between face and car trials) changed over the course of three days. We used single-trial multivariate discriminant analysis of (EEG) data collected from fourteen participants to identify the latency and strength of the early and late components highlighted above (i.e. sensory and decicion evidence, respectively). We found that discrimination performance for the early component remained unchanged during training, whereas that of the late component systematically improved over the three days. Importantly, we also observed that the onset time of the late component, but not the early one, moved earlier in time with learning, consistent with a more efficient representation of decision evidence later in the course of training. Taken together these findings suggest that perceptual learning has a strong influence on the readout of the relevant decision evidence later in the process consistent with previous accounts from the primate literature. Finally, we showed that a simple reinforcement learning (RL) mechanism can account well for the training effects we observed in our data by correlating the trial-by-trial amplitude fluctuations in our EEG components with single-trial decision variables obtained from a RL model.

Disclosures: J. Diaz: None. **M. Philiastides:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.21/BB61

Topic: F.01. Human Cognition and Behavior

Support: Biotechnology and Biological Sciences Research Council (BBSRC; grants BB/J015393/1-2)

Title: Spatiotemporal characteristic of reward-based learning in humans

Authors: *E. FOURAGNAN[1], C. RETZLER[2], K. MULLINGER[3], M. PHILIASTIDES[1]; [1]Inst. of Psychology and Neurosciences, Univ. of Glasgow, Glasgow, United Kingdom; [2]Dept. of Behavioural & Social Sci., University of Huddersfield, United Kingdom; [3]Sch. of Physics and Astronomy, Sir Peter Mansfield Magnetic Resonance Ctr., University of Nottingham, United Kingdom

Abstract: Adaptive decisions depend on accurate outcome representations associated with potential choices. These representations can be acquired with reinforcement learning mechanisms that use the prediction error (PE) - the difference between expected and actual outcomes - as a learning signal to update expectations. PE signals are characterized by their valence and magnitude components; however, the relative contributions and neural systems associated with each component remain debated. Here, we collected simultaneous EEG and fMRI data while participants performed a probabilistic reversal task. Exploiting the trial-by-trial variability in the two modalities we provided a characterization of the global network dynamics associated with PE processing and reward learning in humans. Specifically, we identified two spatiotemporally distinct processing stages of PE valence: an early process driven by an automatic alertness response to negative outcomes and a later, more deliberate, valence assessment, along both outcome types, required for updating value expectations. Importantly, these two valence systems interact to promote switching behavior following negative outcomes. Parallel to the late PE valence evaluation, we found that the brain also represents quantitative information about PE magnitude, in a largely separate network. Intriguingly, we also found an overlap between the late PE valence and magnitude networks in a few nodes. These areas exhibited a response profile consistent with a linear superposition of the two PE components rather than a fully monotonic response as would be expected for a signed PE signal. This finding emphasizes the importance of separate but potentially simultaneous contributions of PE valence and magnitude signals during learning. Crucially, we demonstrate that these findings were missed using conventional model-based fMRI.

Disclosures: E. Fouragnan: None. **C. Retzler:** None. **K. Mullinger:** None. **M. Philiastides:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.22/BB62

Topic: F.01. Human Cognition and Behavior

Support: Biotechnology and Biological Sciences Research Council (BBSRC; grants BB/J015393/1-2)

Title: Spatiotemporal characterization of value-based decision making in humans using simultaneous EEG/fMRI

Authors: *M. PISAURO[1], E. FOURAGNAN[1], C. RETZLER[1,2], K. MULLINGER[3,4], M. PHILIASTIDES[1];
[1]Univ. of Glasgow, Glasgow, United Kingdom; [2]Univ. of Huddersfield, Huddersfield, United Kingdom; [3]SPMMRC, Univ. of Nottingham, Nottingham, United Kingdom; [4]Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Current computational work suggests that value and preference-based decisions involve an integrative mechanism in which subjective value information supporting different decision alternatives accumulates over time to an internal decision boundary. However, the neurobiological validity of this proposition remains unclear. A recent study in humans using electroencephalography (EEG) data suggests this temporal accrual of decision evidence is captured on parietal and frontal electrode sites (Polanía et al., Neuron 2014). However, due to the low-spatial resolution of the EEG the full spatiotemporal characteristics of the decision process could not be inferred. The goal of this study was to couple high temporal resolution, single-trial EEG with simultaneously acquired fMRI to characterize the global network dynamics associated with value-based decisions in humans. Twenty-four subjects participated in the study. EEG and fMRI data were acquired simultaneously on a 64-channel MR-compatible Brain Products EEG system and a 3T Philips Achieva MR scanner, respectively. Subjects performed a two-alternative forced choice task between pairs of snack items, which they have previously rated individually using a 5-point likert scale. Difficulty in the task was controlled by adjusting the overall difference in value between the pairs of items. On average, participants' behavioral performance declined as task difficulty increased (i.e. reaction times increased and accuracy dropped). We fit a sequential sampling model (Ornstein-Uhlenbeck process) to the behavioral data of individual subjects in order to generate predictions on the temporal profile of the underlying accumulating activity. We then correlated these model predictions with the EEG to identify sensor activity mirroring a process of evidence accumulation. Consistent with previous reports, we found two clusters on parietal and frontal electrode sites, which were highly correlated with the temporal dynamics of the decision process as predicted by the model. Finally, we exploited single-trial variability in the slope of the relevant EEG activity in the two clusters of interest to build EEG-informed fMRI regressors to further infer the spatial extent of the brain networks involved in value-based decisions.

Disclosures: **M. Pisauro:** None. **E. Fouragnan:** None. **C. Retzler:** None. **K. Mullinger:** None. **M. Philiastides:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.23/BB63

Topic: F.01. Human Cognition and Behavior

Support: NSF 1460604

NIMH RO1 MH080066-01

NIH R01 NS065046

McDonnell Foundation

Title: Working memory contributions to reinforcement learning: an fMRI study

Authors: *A. G. COLLINS[1], B. A. CIULLO[1], M. J. FRANK[1,2], D. BADRE[1,2];
[1]Brown University, CLPS, Providence, RI; [2]Brown Inst. for Brain Sci., Providence, RI

Abstract: Many cognitive processes contribute to our ability to learn from reinforcement. One such process, referred to as reinforcement learning (RL), relies on the accumulation of reward prediction errors to estimate choice values, and has been related to dopaminergic and striatal function. However, other processes, such as working memory (WM), are also involved in learning. Disentangling their contributions is essential to understanding the role of different neural systems, such as prefrontal cortex, hippocampus and striatum, to human learning. To that effect, we previously developed a task that provided preliminary evidence that WM and RL contributions to learning are supported by prefrontal and dopaminergic striatal function (Collins & Frank 2012). Here we investigate the interactions between the neural processes involved using functional neuroimaging. Using truthful reinforcement, participants learned to pick 1 of 3 actions for each stimulus of a set presented in a given block. To manipulate WM demands and separate the capacity-limited WM system from the RL system, we varied the set size: in each block, participants learned responses for 1 to 6 new visual stimuli. Behavioral results (N = 22) replicated previous findings: subjects learned slower with increasing set-sizes, and their accuracy decreased with the delay between two successive presentations of the same stimulus, two indicators of capacity-limited, short-term working memory. We fit subjects' behavior with our computational model accounting for learning as a mixture of WM and RL, and extracted individual subjects' RL reward prediction errors (RPE) for model-based fMRI analysis. A central question relates to the nature of the interaction between the two systems. If they collaborate, WM should provide expectations that yield lower RPEs in the RL system, and this should be observable in low set sizes or in low delay trials within high set sizes. If they compete, when the WM system dominates in lower set sizes, RPE representations in the RL system should be attenuated for all outcomes. Finally, if RL systems learn independently of explicit memory maintenance, RPE representation should not be sensitive to those factors. fMRI analyses reveal that hippocampus and left intraparietal sulcus were modulated by set size, confirming their role in the WM system. In contrast, inferior frontal sulcus and bilateral ventral striatum were increasingly sensitive to RPE with higher set size. Our results offer preliminary evidence for an

interactive account of RL and WM contributions to learning; subsequent analysis will reveal the nature of the interactions between these dynamic learning systems.

Disclosures: **A.G. Collins:** None. **B.A. Ciullo:** None. **M.J. Frank:** None. **D. Badre:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.24/BB64

Topic: F.01. Human Cognition and Behavior

Support: NSERC Discovery Grant # 34728-58400

Title: Frontal beta oscillations reflect encoding of information related to desired task performance irrespective of feedback valence

Authors: *A. HAJIHOSSEINI, C. B. HOLROYD;
Dept. of Psychology, Univ. of Victoria, Victoria, BC, Canada

Abstract: Several studies have shown that reward feedback stimuli elicit increased beta power (20-30 Hz) over frontal areas of the human scalp. In a previous study, we proposed that frontal beta power reflects the transfer of information about recently rewarded actions from dorsolateral prefrontal cortex (PFC) to brain areas responsible for task execution (SFN, 2014). Here we used a novel guessing task that included on each block of trials separate choice and recall phases to test whether frontal beta power is specifically sensitive to remembering rewarded actions. During the initial choice phase on each block, subjects were required to select 10 stimuli sequentially from a group of 12 stimuli on the screen representing 12 decks of cards. Following each selection, they were presented with either reward (5 cent), error (0 cent), or neutral (1 cent) feedback. Depending on condition, they were given instructions to remember either the rewarded decks (remember reward: RR) or the decks followed by error feedback (remember error: RE), or were not provided with instructions (no-recall: NR). In a subsequent recall phase in the RR and RE conditions, they were asked to select either the previously rewarded (RR) or the error (RE) decks from the previous choice phase. Principle component analysis applied to post-feedback beta power revealed 3 components distributed over the frontal-central, frontopolar, and frontal-lateral areas of the scalp. A 3x3x2 ANOVA with frontal 'virtual channel' (central, polar, lateral), memory condition (RR, RE, NR), and valence (reward, error) on beta power revealed a significant main effect of valence ($F(1,29) = 4.25$, $p = 0.048$) and an interaction between memory condition and valence ($F(2,58) = 3.48$, $p = 0.049$). Post-hoc tests indicated that beta power to reward feedback was larger in the NR and RR conditions compared to the RE condition ($t(29) = 2.8$, $p = 0.01$; $t(29) = 1.9$, $p = 0.06$). Further, beta power to reward and error feedback in the choice phase was negatively correlated with reaction times for correct recalls in the RR ($r = -$

0.37, p = 0.042) and RE (r = -0.57, p = 0.001) conditions, indicating that high beta power in the choice phase predicted faster correct responses in the recall phase, irrespective of the feedback valence. These results point to an important role for frontal beta oscillations in mediating a working memory process elicited by performance feedback irrespective of its valence. In the context of our previous studies, we suggest that this process is elicited by feedback stimuli for the purpose of transferring information about recent sequences of actions maintained in the PFC to downstream areas for task execution according to desired performance.

Disclosures: A. Hajihosseini: None. **C.B. Holroyd:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.25/BB65

Topic: F.01. Human Cognition and Behavior

Support: German Federal Ministry of Education and Research (BMBF) Grants: FKZ 01GQ0913, FKZ 01GQ1313

Title: Different momentums: lifespan age differences in the adaptive regulation of learning rates

Authors: *B. EPPINGER[1], R. BRUCKNER[2], M. R. NASSAR[3], S.-C. LI[1];
[1]TU Dresden, Dresden, Germany; [2]Max Planck Inst. for Human Develop., Berlin, Germany; [3]Brown Univ., Providence, RI

Abstract: Adaptive behavior in uncertain environments depends on the ability to dynamically adjust the rate of learning according to environmental statistics. Results of several previous studies suggest that children and older adults have problems in this type of flexibility. These age-related deficits in adaptive control have been attributed to age differences in the structure and function of the medial prefrontal cortex. However, the computational mechanisms that lead to these deficits are still not well understood. In this study we used a predictive inference task to investigate factors that could contribute to lifespan developmental differences in the adaptive adjustment of learning in a dynamic task environment. Using Bayesian modeling and regression analyses, we dissociated three factors, surprise, uncertainty and reward, which differentially affect learning-rates in children (8-10 years; n=33), adolescents (13-17 years, n=29), younger adults (20-28 years, n=32) and older adults (65-80 years, n=30). We found that children and older adults showed overall reduced learning rates compared to adolescents and younger adults, indicating that they tend to adjust their behavior less to changes in the environment. Furthermore, our results showed that children were particularly sensitive to surprising outcomes (substantial changes in the environment) when compared to the three other age groups. In contrast, in older adults we found a specific deficit in the ability to learn from unsurprising outcomes. Based on

simulations using the computational model we could further show that these deficits are consistent with a diminished capacity to represent uncertainty in older adults. Finally, we found that adolescents were overly sensitive to rewarding outcomes and disproportionately updated their predictions after receiving reward. This reward bias is consistent with the previous literature and was less pronounced in children, younger and older adults. To summarize, our findings point to substantial lifespan developmental differences in the factors that govern adaptive learning in dynamic task environments. It seems conceivable that these developmental changes reflect transformations in the interplay of neuromodulatory systems (such as the locus coeruleus norepinephrine (NE) and the frontal-striatal dopamine (DA) systems) and the medial prefrontal cortex across the human lifespan.

Disclosures: **B. Eppinger:** None. **R. Bruckner:** None. **M.R. Nassar:** None. **S. Li:** None.

Poster

352. Reinforcement and Feedback Learning in Humans

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 352.26/BB66

Topic: F.01. Human Cognition and Behavior

Support: Grant 102-2628-H-002-003-MY3 from the Ministry of Science and Technology in Taiwan

Grant 103-2325-B-002-047 from the Ministry of Science and Technology in Taiwan

Grant support from Drunken Moon Lake Integrated Scientific Research Platform and Aim for Top University Project from National Taiwan University

Title: Effects of affective arousal on choice behavior, reward prediction errors, and feedback-related negativities in human reward-based decision making

Authors: *H.-H. LIU[1,4], M.-H. HSIEH[4], Y.-F. HSU[1,2,3], W.-S. LAI[1,2,3];
[1]Psychology, [2]Grad. Inst. of Brain and Mind Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Psychiatry, Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: Emotional experience has a pervasive impact on choice behavior, yet the underlying mechanism remains unclear. Introducing facial-expression primes into a probabilistic learning task, we investigated how affective arousal regulates reward-related choice based on behavioral, model fitting, and feedback-related negativity (FRN) data. Sixty-six paid subjects were randomly assigned to the Neutral-Neutral (NN), Angry-Neutral (AN), and Happy-Neutral (HN) groups. A total of 960 trials were conducted. Subjects in each group were randomly exposed to half trials of the pre-determined emotional faces and another half of the neutral faces before choosing between two cards drawn from two decks with different assigned reward probabilities. Trial-by-

trial data were fit with a standard reinforcement learning model using the Bayesian estimation approach. The temporal dynamics of brain activity were simultaneously recorded and analyzed using event-related potentials. Our analyses revealed that subjects in the NN group gained more reward values than those in the other two groups; they also exhibited comparatively differential estimated model-parameter values for reward prediction errors. Computing the difference wave of FRNs in reward vs. non-reward trials, we found that, compared to the NN group, subjects in the AN and HN groups had larger "General" FRNs (i.e., FRNs in no-reward trials minus FRNs in reward trials) and "Expected" FRNs (i.e., FRNs in expected reward-omission trials minus FRNs in expected reward-delivery trials), indicating an interruption in predicting reward. Further, both AN and HN groups appeared to be more sensitive to negative outcomes than the NN group. Collectively, our study suggests that affective arousal negatively regulates reward-related choice, probably through overweighting with negative feedback.

Disclosures: H. Liu: None. **M. Hsieh:** None. **Y. Hsu:** None. **W. Lai:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.01/BB67

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R01AG039103

Title: The relationships between age, associative memory performance and the neural correlates of successful associative memory encoding

Authors: *M. A. DE CHASTELAINE[1,2], J. T. MATTSON[3], T. H. WANG[4], B. E. DONLEY[2], M. D. RUGG[2];
[1]Neurobio. & Bahavior, [2]Ctr. for Vital Longevity, Univ. of Texas At Dallas, Dallas, TX; [3]Southwestern Med. Ctr., The Univ. of Texas, Dallas, TX; [4]Dept. of Psychology, Univ. of Texas at Austin, Austin, TX

Abstract: Previous fMRI studies have indicated that encoding-related activity in the inferior frontal gyrus (IFG) is sometimes more lateralized (left > right) in younger relative to older adults, and that greater right frontal activity in older adults is associated with poorer episodic memory performance. In contrast, prior findings also suggest that greater encoding-related hippocampal activity in older adults is predictive of better memory performance. The current fMRI study used an associative recognition procedure to investigate the relationship between episodic memory performance and encoding-related neural activity in young, middle-aged and older adults (total n = 136). Participants were scanned while they made relational semantic judgments on visually presented word pairs and then subsequently made associative recognition

judgments on studied, rearranged (items studied on different trials) and new pairs. Subsequent memory effects were employed to identify successful encoding activity - we focus here on the effects taking the form of greater activity for studied pairs later correctly identified as intact relative to the activity elicited by pairs later endorsed as rearranged. Across groups, subsequent memory effects were identified in a left-lateralized cortical network, including IFG and the hippocampus. There was little evidence that these subsequent memory effects differed with age. Left IFG effects were reliable in all groups while right IFG effects could not be independently identified in any age group. However, IFG effects in the younger group were found to be more lateralized (L > R) than in the older group. In the middle-aged group only, right IFG effects were negatively correlated with later associative memory performance. In both the young and middle-aged groups, right IFG effects were also negatively correlated with an offline measure of verbal memory ability. In contrast, hippocampal subsequent memory effects were positively correlated with later memory performance in all age-groups. The results replicate previous findings in showing greater asymmetry in frontal (IFG) subsequent memory effects in young relative to older adults, and extend prior findings of a negative relationship between right frontal subsequent memory effects and associative recognition in older participants to a middle-aged sample. The findings also replicate a previous report of a positive relationship between encoding-related hippocampal activity and memory performance. In all, the findings suggest that similarities across age groups in the neural correlates of successful associative encoding overshadow any between-group differences.

Disclosures: M.A. De Chastelaine: None. **J.T. Mattson:** None. **T.H. Wang:** None. **B.E. Donley:** None. **M.D. Rugg:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.02/BB68

Topic: F.01. Human Cognition and Behavior

Support: Center for Nutrition, Learning, and Memory at UIUC

Title: Scanpath entropy during study predicts subsequent spatial reconstruction accuracy

Authors: *H. D. LUCAS, P. D. WATSON, J. M. MONTI, E. MCAULEY, A. F. KRAMER, N. J. COHEN;
Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The ability to encode complex information given limited time and resources is an important cognitive skill. Previous work has implicated hippocampal-prefrontal and

hippocampal-striatal brain networks in guiding advantageous study decisions via environmental exploration. However, little is known about how eye movement patterns relate to the ability to optimize intake of currently visible information. We recorded eye movements while a group of 78 healthy elderly participants studied a series of displays, each consisting of several objects in varying arbitrary spatial configurations. Participants were given 20 seconds to study each display, which they then attempted to reconstruct from memory. We found that participants' scanpath entropy during study- a measure of the randomness or unpredictability in their item-to-item fixation patterns- showed a significant negative relationship to subsequent reconstruction accuracy. In particular, participants who engaged in higher-entropy (e.g more random) scanning patterns showed a greater tendency to later "swap" or reverse the relative positions of objects within the array. Importantly, scanpath entropy while studying the displays also showed a significant negative relationship to performance on a separate auditory test of delayed story recall, suggesting that this measure may reflect an ability to self-organize information that extends to multiple stimulus domains and modalities. Finally, participants who reported more depressive symptoms also showed higher scanpath entropy, perhaps as a result of changes to the dopaminergic hippocampal-VTA circuit. While depressive symptoms did not directly correlate with memory performance, including entropy as a mediator revealed indirect effects of depressive symptoms (mediated by entropy) on both later spatial reconstruction accuracy and delayed story recall. These data suggest that scanpath entropy constitutes a useful tool with which to quantify the organization of strategic viewing behaviors, and may also provide a window into alterations to such behaviors in neurological and psychiatric disorders.

Disclosures: **H.D. Lucas:** None. **P.D. Watson:** None. **J.M. Monti:** None. **E. McAuley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Abbott Nutrition. **A.F. Kramer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Abbott Nutrition. **N.J. Cohen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Abbott Nutrition.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.03/BB69

Topic: F.01. Human Cognition and Behavior

Title: Familiarity-novelty detection is an intrinsic property of cortical microcircuits with NMDA receptor-dependent synaptic plasticity

Authors: *X. ZHANG[1], H. JU[3], T. B. PENNEY[2], A. M. VANDONGEN[3];
[1]NUS Grad. Sch. for Integrative Sci. and Engin., [2]Psychology, Natl. Univ. of Singapore,

Singapore, Singapore; [3]Neurosci. & Behavioral Disorders Program, Duke-NUS Grad. Med. Sch., Singapore, Singapore

Abstract: Humans instantly recognize a previously seen face as "familiar" and are capable of recognizing thousands of faces. The ability to rapidly assess whether a sensory input is familar or novel is not restricted to faces and does not appear to require a conscious effort. To investigate the mechanisms underlying this familiarity-novelty detection ability we used a neural network model of cortical microcircuits consisting of spiking neurons with random recurrent connections, following the "liquid state machine" paradigm. To allow for unsupervised learning, we introduced NMDA receptor-dependent synaptic plasticity processes. Synaptic weights are initially set to random values and are subsequently altered by sensory experience through LTP, LTD and STDP processes. In the experiments we presented images of human faces, dog faces and car fronts to networks of 2,000-15,000 spiking neurons organized in a 3D topology. To facilitate analyzing the effects of plastic changes, overall pixel intensity levels of the images were adjusted so that the cumulative firing rate was the same before learning. Network activity evoked by the image stimuli altered synaptic efficacy, which in turn resulted in the network responding more strongly to a previously seen image. As a result, the networks distinguished familiar from novel images in their overall firing rate. Small networks of 2,000 spike neurons were able to encode the features of a single human face image through unsupervised learning and identifiy it accurately out of a set of 30 test images. Large networks of 15,000 spiking neurons were able to learn and memorize multiple faces simultaneously with high accuracy. Some of the best-performing networks could memorize 60 human faces. These results indicate that small cortical microcircuits containing NMDA receptor-dependent synaptic plasticity have an intrinsic ability for detecting whether a sensory input is familiar or novel. This abilty does not require a specialized wiring diagram or supervision, and therefore can be expected to emerge naturally in developing cortical circuits.

Disclosures: X. Zhang: None. **H. Ju:** None. **T.B. Penney:** None. **A.M. VanDongen:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.04/BB70

Topic: F.01. Human Cognition and Behavior

Support: SINAPSE (www.sinapse.ac.uk)

Title: Episodic memory is affected by emotional valence, not just arousal: Evidence from behaviour and Event-Related Potentials

Authors: *G. MACKENZIE, D. I. DONALDSON;
Psychology, Univ. of Stirling, Stirling, United Kingdom

Abstract: Research shows that emotion can enhance episodic memory, and amygdala activity projecting to the hippocampus is likely to be responsible. Emotionally arousing stimuli generate more amygdala activity than neutral stimuli and are therefore encoded and then retrieved preferentially in the hippocampus. There are, however, two principal dimensions of emotion - arousal and valence - and the amygdala-hippocampus model only provides an adequate account of how arousal affects memory. In practice, most research conflates the effects of arousal and valence on memory, but independent effects of valence on source memory have been reported, with behavioural impairment for negative but not positive pictures (MacKenzie, Powell & Donaldson, 2015). Here, we ask whether the neural basis of episodic memory is sensitive to emotional valence. Participants performed a source memory task in which the emotional valence (negative/positive) of stimuli was manipulated while arousal was equated across conditions. Pictures were studied with either a square or circle pattern superimposed on the image. Later, participants discriminated between studied and new pictures, making circle/square source judgments for pictures that were recognised. Source memory performance was better for positive than negative pictures. Furthermore, Event-Related Potential (ERP) old/new effects from 500-800msec post-stimulus onset had different scalp distributions for negative and positive pictures. Negative pictures were associated with a left parietal old/new effect, which is typically interpreted as a neural correlate of recollection. By contrast, positive pictures were associated with a more broadly distributed effect, extending to sites over frontal cortex. This pattern of ERP results indicates that negative and positive pictures are recollected differently, and that the superior source memory performance for positive pictures is linked to recruitment of additional retrieval processes. Most importantly, the enhanced source memory observed for positive pictures occurred despite any differences in arousal between the sets of pictures. These findings show that effects of emotion on episodic memory cannot be reduced to effects of arousal as the amygdala-hippocampus model suggests. Rather, the emotional valence of stimuli can affect episodic memory directly, at least during performance of source memory tasks.

Disclosures: G. Mackenzie: None. **D.I. Donaldson:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.05/BB71

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 5R01MH072966

Title: Prestimulus parahippocampal/hippocampal activity predicts associative retrieval success

Authors: *K. L. VILBERG, M. D. RUGG;
Ctr. for Vital Longevity, Sch. of Brain & Behavioral Sci., Univ. of Texas, Dallas, Dallas, TX

Abstract: While there exists a growing literature on how prestimulus fMRI activity at encoding relates to subsequent memory performance, to our knowledge, no prior study has evaluated whether prestimulus fMRI activity at test differs according to retrieval success. We conducted two experiments in which recollected information had to be maintained in working memory prior to a behavioral response. In both experiments, word-image pairs were studied in an intentional encoding task. In one experiment, words served as test items, and in the other experiment, images served as test items. Participants were instructed to try to recall the paired associate of the test item and hold it in mind until a question appeared at which time they were to answer the question with respect to the retrieved information, or if not recollected, to respond 'old, associate not recollected' or 'new'. In each case, a variable fixation preceded test item onset, allowing pre-stimulus, stimulus, and delay-related activity to be deconvolved. In the current analysis, we modeled the onset of the prestimulus fixation character, the onset of the test item onset, and a variable duration maintenance interval in order to distinguish between brain regions exhibiting recollection effects according to their time courses. In line with our prior findings in which the prestimulus fixation was not explicitly modeled, a large network of lateral cortical regions exhibited sustained recollection effects, whereas regions such as medial prefrontal cortex, retrosplenial cortex/posterior cingulate, and parahippocampal cortex displayed transient positive recollection effects. Prestimulus recollection effects were identified in bilateral parahippocampal cortex, extending into the posterior hippocampus. In each cluster, activity associated with test item recognition in the absence of associative retrieval exceeded that for trials associated with successful associative retrieval prior to stimulus onset and until roughly 3.5s thereafter at which point these differences reversed direction. The results raise the possibility that a reduction of ongoing activity in the parahippocampal cortex/posterior hippocampus prior to the onset of a retrieval cue facilitates associative retrieval.

Disclosures: K.L. Vilberg: None. **M.D. Rugg:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.06/BB72

Topic: F.01. Human Cognition and Behavior

Support: The I-CORE Program of the Planning and Budgeting Committee and The Israel Science Foundation (grant No. 51/11)

Title: Associative activation and its relation to exploration in the brain

Authors: *S. BAROR, M. BAR;
Bar- Ilan Univ., Ramat Gan, Israel

Abstract: We tend to think about associative activation as an automatic process, whereby if A is associated with B, B is activated every time A is activated. Sometimes the activation of associated concepts depends on context (e.g., a towel in the shower and the same towel on the beach is associated with different items), but even then it is possible that irrelevant associations are still being activated, at least briefly. Here we challenged this implicit assumption of automaticity in associations by manipulating load. In a series of 4 experiments, participants responded in a free association task while load could be either high or low. Three types of load were employed: working memory load, cognitive load unrelated to working memory, and perceptual load. In all experiments we found that the associations provided by participants under the low-load conditions were significantly more diverse than the high consensus obtained under high load. In other words, with less load participants seem to activate more remote associations. These findings imply that high load has an inhibitory effect on active resources invested in the retrieval of associations. It is suggested that, by default, activation of associations is an active process of exploration, which under high load is narrowed to the immediate associations. Implications to exploration vs. exploitation in the brain, as well as to mood disorders and creativity will be discussed.

Disclosures: S. Baror: None. **M. Bar:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.07/BB73

Topic: F.01. Human Cognition and Behavior

Support: NIH F32 HD 078223

Title: An optimal slow-wave phase for learning-related auditory cues during sleep to improve subsequent memory performance

Authors: *L. BATTERINK, J. CREERY, K. A. PALLER;
Northwestern Univ., Evanston, IL

Abstract: Slow-wave sleep is characterized by synchronized EEG activity appearing as high-amplitude slow waves (0.5-4 Hz). These slow waves correspond to the alternation of active (Up) and quiescent (Down) states in cortico-thalamic networks. The Up state is a period of excitation, in which cortical neurons are depolarized and likely to fire action potentials; the Down state is a period of inhibition, in which neurons are hyperpolarized and unlikely to fire. Slow oscillations are known to play an important role in memory consolidation, presumably coordinating network

interactions (e.g., hippocampal-cortical and cortico-cortical). Research using a technique known as targeted memory reactivation (TMR) during slow-wave sleep shows that sensory stimulation with cues associated with previous learning can enhance memory consolidation through reactivation of individual item memories. In the present study, we hypothesized that the phase of the slow oscillation at which a learning-related cue is presented (i.e., corresponding to Up versus Down states) would influence whether the associated memory would be strengthened. In two separate studies, participants were taught arbitrary associations (picture-location or picture-word). During learning, each picture was also paired with a related environmental sound (e.g., cat with meow). Memory was assessed with an associative recall test. During an afternoon nap, half of the sounds were presented at a low intensity. After awakening, each association was tested again. Consistent with our hypothesis, slow-wave phase prior to sound onset predicted the extent to which memory strength changed. Memories that were subsequently strengthened versus subsequently weakened showed opposite phases for slow waves during the pre-stimulus period. These results suggest that TMR stimuli are more likely to be processed and trigger memory reactivation when they occur at the optimal phases of a slow oscillation. This optimal phase may correspond to the cortical Up state, enabling communication and synchronization across brain regions. These findings provide insights into the mechanisms underlying spontaneous memory reactivation during sleep, suggesting that reactivation is most likely to occur during cortical Up states.

Disclosures: **L. Batterink:** None. **J. Creery:** None. **K.A. Paller:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.08/BB74

Topic: F.01. Human Cognition and Behavior

Support: National Institute of Mental Health Grant R01MH60941 (to D.L.S.)

Title: Meta-analysis specifying the core-network supporting episodic memory and episodic simulation

Authors: *R. G. BENOIT, D. L. SCHACTER;
Psychology, Harvard Univ., Cambridge, MA

Abstract: It has been hypothesized that our capacity to simulate hypothetical episodes is based on an episodic memory system that provides access to stored episodic details and the constructive processes to recombine these details into novel episodes. Key evidence for this constructive episodic simulation hypothesis has been provided by a number of neuroimaging experiments, which indicate that both episodic simulation and episodic memory are supported by

the same core network of brain regions. Here, we employed Activation Likelihood Estimation (ALE) to quantitatively summarize the results of those experiments. Specifically, we only included experiments that formally tested for spatial overlap of episodic memory and episodic simulation (e.g., by employing a conjunction approach), thus providing strict evidence for the core-network. At the same time, we included studies that examined the simulation of a variety of different hypothetical episodes (i.e., possible personal future, fictitious, and counterfactual past episodes), thus increasing the generalizability of our results. Our meta-analysis revealed the expected convergence of activation within the medial temporal lobes, large parts of the medial surface as well as parts of the lateral temporal and inferior posterior parietal cortices. In addition, episodic simulation and memory both consistently recruited a few other regions including parts of the bilateral dorsolateral prefrontal cortex. All of the identified regions overlapped with the default network. Episodic simulation - compared with episodic memory - presumably further requires stronger engagement of some parts of the core network (e.g., for the retrieval of disparate episodic details) and also recruitment of additional brain regions (e.g., for the novel recombination). A second ALE analysis indeed identified regions that were consistently more strongly engaged during episodic simulation than episodic memory. These included parts of the core network in the left dorsolateral prefrontal cortex and inferior posterior parietal lobe in addition to auxiliary regions that were broadly distributed across the default and fronto-parietal control networks. Together, the analyses quantitatively specify the set of regions engaged during episodic simulation, thus providing a foundation for further inquiries into their interactions and specialized contributions.

Disclosures: R.G. Benoit: None. **D.L. Schacter:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.09/BB75

Topic: F.01. Human Cognition and Behavior

Title: The influence of mental countermeasures on memory detection using an fMRI-based Concealed Information Test

Authors: *J. PETH[1], T. I. BROWN[2], A. D. WAGNER[2], M. GAMER[1];
[1]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Dept. of Psychology and Neurosciences Program, Stanford University, CA

Abstract: The Concealed Information Test (CIT) is an implicit psychophysiological technique that aims to detect memory for crime details based on multiple-choice questions including crime related and equally plausible neutral answer options. Previous CIT studies revealed increased neural activity in a ventral frontal-parietal brain network (i.e. bilateral inferior frontal gyrus, right

middle frontal gyrus, right temporoparietal junction) for the comparison between crime details and neutral details. Countermeasures (CM) are techniques to voluntarily manipulate physiological responses during the CIT which might challenge the test's validity and its application in real life (e.g. courtroom) settings. Physical CM have been shown to strongly decrease validity of an fMRI-based CIT for autobiographic information. The impact of mental CM on neural activation during the CIT is unclear. To close this gap, the current mock crime study compared three groups of subjects. After completing a mock crime, the "guilty" subjects either attended the CIT without previous training (standard guilty group, n = 20) or following a detailed mental CM training (CM group, n = 20). The mental CM strategy involved imagining an emotional scene for half of the neutral answer options during the CIT in order to increase physiological similarity to remembering crime details. The CM details were memorized by the CM group prior to the test. Finally, uninformed innocents (innocent group, n = 20) completed the same CIT without any knowledge about the mock crime. All three participant groups performed the CIT while undergoing fMRI scanning, and univariate and multivoxel pattern classification analyses (MVPA) were used to detect brain activity distinguishing crime and neutral details. For the standard guilty group, univariate analyses demonstrate increased activity for crime details in the expected frontal-parietal brain regions and enabled a valid differentiation from innocent subjects. However, these regions were also activated for neutral details when employing mental CM in the CM group and decreased the CIT's validity to chance level. MVPA results demonstrate that the pattern classifier is still able to differentiate between crime details and CM details, and distinguish each of them from neutral details. Thus, recognition of crime details and practiced CM details seems to activate partly different brain regions. The current findings replicate the successful detection of memory for crime details in guilty subjects. In addition, our data reveal potential limitations of CIT applications in real life but we also found some evidence for successful detection of mental CM usage.

Disclosures: **J. Peth:** None. **T.I. Brown:** None. **A.D. Wagner:** None. **M. Gamer:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.10/BB76

Topic: F.01. Human Cognition and Behavior

Support: JSPS Grant-in-Aid for scientific research (S) # 22220003.

Title: Spatial cueing biases recognition memory judgments

Authors: ***K. MIYOSHI**, H. ASHIDA;
Kyoto Univ., Kyoto, Japan

Abstract: Different researchers have reported that the processing fluency of the stimulus significantly affects recognition memory judgments. In the present study, the effect of processing fluency induced by spatial cueing on recognition memory judgments was assessed. Participants memorized pictures of everyday objects, and their spatial attention was manipulated in a Remember/Know recognition memory test. Stimulus location was either predicted (congruent condition) or unpredicted (incongruent condition) using an arrow cue. The results revealed that participants exhibited familiarity-based false recognition more frequently in the incongruent condition. This increased false recognition was associated with participants' liberal response bias, not with decreased response accuracy. In the incongruent condition, participants may have attributed part of the perceived disfluency to the spatial cue and overestimated the fluency for the stimulus, leading to increased false recognition. In contrast, in the congruent condition, participants may have attributed some parts of the perceived fluency to the spatial cue and underestimated the fluency for the stimulus, leading to decreased false recognition. In short, stimulus-irrelevant spatial cueing induces unintentional reasoning about the source of fluency and biases familiarity-based recognition memory. The present results have significant implications for everyday situations, in that the incidental manipulation of attention could occur outside the laboratory. Possibly, déjà vu might be one manifestation of the effect reported here.

Disclosures: K. Miyoshi: None. **H. Ashida:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.11/BB77

Topic: F.01. Human Cognition and Behavior

Support: EU FP7 (Grant agreement 604102)

Foundation Adelis

Howard Hughes Medical Institute

We are grateful to M. Kahana and his University of Pennsylvania research group for making their raw data publicly available

We thank Prof. Nachum Ulanovsky for critical comments

Title: Practice makes perfect in memory recall

Authors: *M. KATKOV[1], S. ROMANI[2], M. TSODYKS[1];
[1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Human memory is able to store vast amounts of information, however retrieval of this information when no cue is provided is challenging. For instance, in the classical free recall paradigm, participants are requested to recall words from randomly assembled lists in an arbitrary order. Typically, they cannot recall complete lists of more than 5 words. Nevertheless, there is a large variability in the number of recalled words both within and across participants. Here we asked whether individual differences in performance result from different patterns of recall, and whether there is an effect of practice. Analysis of the large dataset of free recall data collected in the lab of M. Kahana (UPenn) revealed a small fraction of participants that were able to recall complete lists of 16 words ("perfect trials") in many trials. Inspection of the recall patterns of these participants revealed that they widely apply one of 4 positional grouping strategies: "forward chaining" - recall of words in the order they were presented; a number of positional chunking where words from one chunk (a set of consequitively presented words) are recalled before any words from another chunk. In particular, we identified "4444", "33334" and "43333" chunking, where each digit represents the number of consequitive words in a chunk. We quantified the extent of positional grouping applied by participants in each trial. In trials with strong positional grouping, we observed that the average time to recall a new word is substantially smaller (and was approximately constant across output positions) when next recalled word is within the same chunk than when it is from a different chunk. The average performance of all participants was modestly increasing for the first 4 days, saturating afterwards. All participants showed increase in the number of perfect trials, but for the high performance participants this increase was especially pronounced - from few percent initially towards 30% during last day, whereas the rest of participants showed increase from 1% to 6%. Also the increase in the extent of positional grouping was significantly higher for high performance participants compared to the rest. Our results indicate that practicing free recall of randomly assembled lists cannot substantially improve the performance for most people, possibly due to hard limitations arising from random encoding of words in long-term memory; utilizing positional strategies could be the only efficient way to improve the recall but only a small fraction (around 10 percent) of participants could acquire them on their own with single exposure to each list.

Disclosures: **M. Katkov:** None. **S. Romani:** None. **M. Tsodyks:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.12/BB78

Topic: F.01. Human Cognition and Behavior

Title: Competitive effects of pre-existing and new memory traces during reconsolidation of stimulus-response associations

Authors: *A. RICHTER[1], M. GUITART-MASIP[2], A. BARMAN[1], C. SEIDENBECHER[1], B. SCHOTT[1];
[1]Leibniz Inst. For Neurobio., Magdeburg, Germany; [2]Aging Res. Center, Karolinska Inst., Stockholm, Sweden

Abstract: The term reconsolidation refers to a process in which consolidated memories are reactivated and thereby become labile and can be modified before getting consolidated again. Depending on the treatment (e.g. injection of a drug, behavioral manipulations) and different parameters (e.g. memory types prone for reconsolidation, characteristics of successful reactivation), that are currently being investigated, the memory trace can be strengthened, updated or weakened. The aim of our study was to elucidate neuropsychological and brain mechanisms of reconsolidation processes in human probabilistic stimulus-response (S-R) association learning. To this end we designed a task including a probabilistic learning, a relearning and a retrieval session over three consecutive days. On the first day, participants learned S-R assciations that were reconfigured on the second day. Participants who reactivated the initial S-R pairings before relearning showed improved memory for the original associations from day 1, but impaired memory for the reconfigured associations from day 2. On the other hand, participants who did not reactivate the original associations did not exhibit such a pattern. Furthermore the effect of reactivation vanishes, when the relearning (day 2) is prolonged. These results suggest that reactivating memory for previously learned probabilistic S-R associations can induce a competition of pre-existing and newly acquired memory traces. Currently we are planning to investigate the neural correlates of the competitive effects of pre-existing and new memory traces during reconsolidation.

Disclosures: A. Richter: None. **M. Guitart-Masip:** None. **A. Barman:** None. **C. Seidenbecher:** None. **B. Schott:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.13/BB79

Topic: F.01. Human Cognition and Behavior

Title: An individualized approach to treatment of prospective memory in people with brain injury

Authors: *E. AIKEN, Z. BITAN, S. RASKIN;
Trinity Col., Hartford, CT

Abstract: Three case studies of Kosakoff's syndrome, meningioma, and traumatic brain injury (TBI), will be presented to illustrate the variety of cognitive deficits across different individuals

with ABI. This study uses cognitive rehabilitation therapies to target individual cognitive symptoms. Results and discussion place emphasis on the use of prospective memory (PM) training for treating Kosakoff's syndrome. The Memory for Intentions Screening Test (MIST) served as the assessment for PM analysis pre and post rehabilitation. The data from this study will be used as a model for a larger study analyzing the effectiveness of different cognitive rehabilitative therapies: PM training, attention process training (APT) and executive function training, in treating ABI that are individualized based on cognitive symptoms.

Disclosures: **E. Aiken:** None. **Z. Bitan:** None. **S. Raskin:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.14/BB80

Topic: F.01. Human Cognition and Behavior

Support: ERC Starter Grant number 337822 "TRANSMEM"

Title: Stable representations of lifelike events over the course of one week

Authors: ***C. OEDEKOVEN**, J. KEIDEL, S. BERENS, C. M. BIRD;
Sch. of Psychology, Univ. of Sussex, Brighton, United Kingdom

Abstract: Episodic memory for events is a crucial part of our everyday lives. While we experience events we combine incoming information with our prior knowledge to create coherent memories. The active rehearsal of these memories further stabilizes them and thus improves later recall (Roediger and Karpicke, 2006). The aim of our fMRI study was to investigate the stability of memories for lifelike events over the course of a week. We report results in a group of 21 young adults (aged 25.6 years, range 18-35, 11 female) who were scanned twice. During their first scan participants encoded and silently recalled 24 short video clips. Vividness was rated after each recall. Following a 7 day delay, they silently recalled all 24 videos again in the scanner and then described the videos afterwards. Univariate analyses were conducted using SPM8 and whole- brain searchlight representational similarity analyses (RSA) using the CosmoMVPA toolbox. Participants recalled 11.5 details from each video after one week, whilst vividness ratings for the recall phases dropped by 20%. RSA identified a network of regions including posteriomedial cortex, bilateral parahippocampal gyri, bilateral temporoparietal junction and right anterior temporal lobe where patterns of activity during encoding correlated with immediate recall of the same video. A strikingly similar network of regions was found when comparing patterns of activity during immediate recall and recall of the same videos a week later. Interestingly, within the left dorsal medial frontal lobe, the strength of representational similarity between immediate and delayed recall correlated with the amount of

detail recalled for each video. In the univariate analyses, this region showed higher activity during both recall phases but not encoding. Overall, we identified a stable network of regions that showed very similar patterns of activity during encoding, immediate recall and delayed recall, despite the fact that ratings of vividness decreased over the course of a week. This shows that during encoding, participants created a coherent representation of the content of each video which was reinstated within the network at both immediate and delayed recall. Since the events within each video took place in multiple locations, this representation cannot simply reflect spatial context but instead must include a more abstract representation of its narrative. This interpretation is further supported by the correlation of dorsal medial frontal lobe activity with the amount of detail recalled and is consistent with the proposal that these regions integrate episodic information with prior semantic knowledge (Binder et al., 2009).

Disclosures: **C. Oedekoven:** None. **J. Keidel:** None. **S. Berens:** None. **C.M. Bird:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.15/BB81

Topic: F.01. Human Cognition and Behavior

Support: NIH grant NS079698

Title: Post-encoding theta-burst TMS to lateral occipital cortex impairs associative memory retention

Authors: ***A. TAMBINI**, M. D'ESPOSITO;
Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA

Abstract: The formation of stable long-term memories is thought to be dependent on both brain activity during the initial encoding of an event, but also on processes that unfold during post-encoding time periods. In addition to the importance of sleep, recent work has suggested that awake rest periods immediately after encoding may play a role in memory consolidation. Here, we sought to casually test the role of post-encoding rest periods in memory by asking whether post-encoding transcranial magnetic stimulation (TMS) applied to a cortical site involved in stimulus processing is sufficient to 1) impair memory retention and 2) disrupt putative consolidation-related activity during post-encoding rest. To address these questions, participants performed an incidental object-face associative encoding task during fMRI scanning, which has previously been shown to influence patterns of post-encoding resting connectivity. The encoding task was preceded by BOLD and perfusion ASL rest scans. Immediately after encoding, participants performed a brief (~10 minute) memory test followed by continuous theta-burst TMS (TBS) to either the posterior portion of the lateral occipital complex (LO, involved in

object processing) or a control site (primary somatosensory cortex, S1) and a series of BOLD and perfusion rest scans lasting for approximately 40 minutes. Participants returned to perform delayed memory tests approximately 2 and 24 hours after TMS. The retention of both associative and item memory was measured between the initial memory test and delayed memory tests. Associative memory retention across a two-hour delay was significantly reduced when TBS was applied to LO versus the control site and a behavioral control group that did not undergo TMS. In contrast to associative memory, measures of item memory retention did not differ as a function of TMS site. Experience-related increases in regional cerebral blood flow (rCBF) from pre- to post-encoding rest were found in the control group in LO and the hippocampus, but these increases were not present when TBS was applied to LO. These findings suggest that interfering with brain activity within a window of approximately 10 minutes to 1 hour after encoding with TBS is sufficient to impair later associative memory performance. More broadly, these data highlight the potential role of post-encoding rest periods in associative memory retention.

Disclosures: A. Tambini: None. **M. D'Esposito:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.16/BB82

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant RO1EY024056

Title: How is conceptual knowledge represented in the temporal pole? A cross-modal perspective and familiarity effects

Authors: *L. T. LIKOVA[1], C. W. TYLER[2], K. MINEFF[2], S. NICHOLAS[2];
[1]Smith-Kettlewell Eye Res., San Francisco, CA; [2]Smith-Kettlewell Eye Res. Inst., san Francisco, CA

Abstract: How is conceptual knowledge represented? Do the temporal poles (TP) form a unified bilateral representational system for conceptual knowledge, or is there instead left/right specialization? To address this controversial issue and its generalization to non-visual modalities, we took a novel cross-modal approach in a brain imaging study of writing and drawing from conceptual memory in blind participants. Methods: FMRI was conducted in a Siemens 3T scanner with a custom tablet system for the recording of haptic output. The conceptual information was presented tactilely through reading of a Braille text, and its retrieval from memory was expressed through two non-visual modes - Braille writing and blind drawing. Blind participants read Braille paragraphs describing objects, faces, scenes and navigation sequences, then expressed their comprehension of each by i) non-visual (Braille) writing-from-memory, and

ii) non-visual drawing-from-memory (20s/task). To assess familiarity effects, each form of output was repeated a second time after a 20 sec delay. Results/Conclusions: Two adjacent subdivisions of the temporal pole - the apex (TPa) and a dorso-medial region (TPdm) showed contrasting behavior as a function of task-domain and familiarity. TPdm responses appeared only in the familiarity (second) phase for both Braille writing and drawing. Comprehension of the Braille text expressed through the Braille writing-from-memory task produced inter-hemispheric push-pull behavior with a strongly left-lateralized response in both TPa and TPdm, combined with extensive contralateral suppression. In contrast, however, the blind drawing-from-memory task showed a remarkably different behavior of fully bilateral responses in both areas with no inter-hemispheric push-pull, thus conforming to the bilateral TP model for conceptual knowledge representation. The familiarity assessment, on the other hand, revealed a profound inter-region push-pull of the bilateral drawing signals, being dominant in TPa in the unfamiliar phase, switching to dominating in TPdm in the familiar phase, thus revealing a previously unexplored form of familiarity dissociation. Conclusions. This first Braille writing and Braille-derived drawing study thus reports a distinctive form of hemispheric specialization and differential familiarity effects in the TP. These results indicate a strong task-domain dependency of the localized functional architecture in the TP, which may explain some of the controversies in the literature. Overall, the current findings extend the study of conceptual knowledge representation beyond the visual modality for both encoding and retrieval.

Disclosures: **L.T. Likova:** None. **C.W. Tyler:** None. **K. Mineff:** None. **S. Nicholas:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.17/BB83

Topic: F.01. Human Cognition and Behavior

Title: Simulations on the effect of external semantic interference in lexical retrieval and priming in memory

Authors: *R. CHANDRAMOULI[1], K. HATALIS[2];
[1]Temple/ St. Luke's Med. Sch., Bethlehem, PA; [2]Lehigh Univ., Bethlehem, PA

Abstract: Abstract: *Background:* Memory retrieval is partly based on semantic organization and response times based on priming. Once an object has been presented in memory multiple times, it is considered to be primed for rapid retrieval. During this priming process, semantic memory plays an important role. By observing objects with similar semantic features we can create an organized “thesaurus” of an object so it can be easily retrieved from memory. This would include trucks, trains, and boats, in the example of a car, which are all modes of transportation. When this primed object of a car is retrieved from memory, it will initially collect

all objects within the same semantic framework. During this incremental learning process there are interfering effects for retrieval known as cumulative semantic interference. Interference occurs when there is an impaired ability to remember an item when it is similar to other items stored in memory. When a person names a picture under the blocked-cyclic naming paradigm, they would take longer to recall an object when they observe repeated pictures from the same semantic category. We highlight the hypothesis that words with similar phonemes, such as a penguin and puffin, have amplified interference that occurs during lexical retrieval. *Methods:* We present a two-layer neural network with a distributed semantic input layer and a lexical output layer with added neurons for external activations to analyze the phonological influence on lexical retrieval and priming. Homogeneous and heterogeneous word test sets were created with semantic only, phonological only, and both semantic phonological similarities. *Results:* Our computer model provides a mapping for retrieval pathways and interference patterns during memory retrieval. Results show that retrieval based on mean selection time per naming cycle is delayed for homogeneous sets when sequential items presented to the network contain both semantic and phonological similarities vs only semantic. *Conclusion:* We examine through simulations that semantic interference is further exacerbated by the same phonemes among two words. For neurological conditions involving memory, the idea of external semantic activation and interference using computer models can assist in research by modulating further understanding about learning processes and ways of improving memory retrieval outcomes.

Disclosures: R. Chandramouli: None. **K. Hatalis:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.18/BB84

Topic: F.01. Human Cognition and Behavior

Support: ERC Grant 337822 “TRANSMEM”

ESRC

Title: Configural learning engages the semantic memory system but generalisation involves the hippocampus

Authors: *S. C. BERENS, C. M. BIRD;
Univ. of Sussex, Brighton, United Kingdom

Abstract: Configural learning entails forming a conjunctive representation of individual stimuli. Structural learning, a configural learning subtype, additionally specifies the temporal or spatial arrangement of stimuli. While non-structural configural learning may be subserved by the perirhinal cortex, lesion studies in rodents suggest that structural learning is dependent on the

hippocampus. The hippocampus has also been implicated in generalising previously acquired knowledge including learned configural information (e.g. Eichenbaum & Cohen, 2001). In the present fMRI study we investigate the brain regions involved in configural learning and generalisation. Fifteen right-handed participants (aged 18-35; 8 females) engaged in a virtual reality trial-and-error learning task with spatial-structural and non-structural trials. To investigate the generalisation of configural information post-acquisition, stimuli from structural trials were rearranged to produce a test of discrimination transitivity. We observed monotonic increases in BOLD activity coincident with both structural and non-structural memory acquisition in the inferior temporal lobes (bilaterally), left angular gyrus and basal forebrain (bilaterally). Contrary to predictions, no areas within the medial temporal lobes (including the hippocampus and perirhinal cortex) showed overall levels of activity that correlated with learning or differentiated between structural and non-structural trial types. Nonetheless, a multivoxel pattern analysis demonstrated that the hippocampal, parahippocampal, perirhinal, and entorhinal cortices did contain trial relevant information throughout the task. Moreover, the strength of this information in early trials predicted subsequent behavioural performance in the final trials. Lastly, during the generalisation test, behavioural performance of individual participants positively correlated with their BOLD activity in the right posterior hippocampus. We suggest that participants perform this task using their semantic memory system; new configural representations are created in the inferior temporal cortex and selection between competing similar representations is supported by the angular gyrus. This is consistent with these regions known roles in semantic memory storage and semantic control respectively. By contrast, the hippocampus is likely to be required when initially forming these representations and then generalising the information to novel situations. This supports the proposed role of the hippocampus in flexibly expressing newly formed memories on novel situations.

Disclosures: S.C. Berens: None. **C.M. Bird:** None.

Poster

353. Human Memory: Episodic and Semantic Memory Processes

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 353.19/BB85

Topic: F.01. Human Cognition and Behavior

Title: Relationship between clinical and electrophysiological measures of prospective memory in individuals with brain injury

Authors: *S. A. RASKIN, C. PEDRO, E. AISENBERG, T. BLOOMQUIST, M. RACE, N. KAUR;
Trinity Col., Hartford, CT

Abstract: Prospective memory (PM) involves the ability to form and realize intentions after a time delay (Einstein & McDaniel, 1990). This experiment aims to examine the relationship between clinical measures of PM and an event-related potential paradigm (West & Ross-Munroe, 2002). Electrophysiological data was collected while performing a computerized laboratory PM measure and was compared to a clinical measure, the Memory for Intentions Screening Test (MIST) (Raskin, Buckheit, & Sherrod, 2011) in healthy adults (HA), individuals with severe acquired brain injury (sABI) and mild acquired brain injury (mABI). Results revealed that individuals with sABI performed significantly worse than individuals with mABI and HA on all variables of the MIST. Results also showed reduced amplitudes in individuals with sABI, on ERPs that have been associated with intention formation and intention retrieval when compared to individuals with mABI and HA. In addition, total score on the MIST was related to variables associated with attention retrieval. Overall, these findings suggest that individuals with sABI have deficits in PM compared to individuals with mABI and HA and that the MIST may be a valid measure of underlying brain processes in PM.

Disclosures: S.A. Raskin: None. **C. Pedro:** None. **E. Aisenberg:** None. **T. Bloomquist:** None. **M. Race:** None. **N. Kaur:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.01/BB86

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant K12 HS023011

Title: Neuroanatomical evidence for a multidimensional representation of impulsivity: a voxel-based lesion symptom mapping approach

Authors: *V. MANDOSKE[1], A. CHAU[1], K. K. HAUNER[1,2,3], F. KRUEGER[4,5], J. GRAFMAN[1,2,3];
[1]Cognitive Neurosci. Lab., Rehabil. Inst. of Chicago, Chicago, IL; [2]Dept. of Physical Med. and Rehabil., [3]Dept. of Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [4]Mol. Neurosci. Dept., [5]Dept. of Psychology, George Mason Univ., Fairfax, VA

Abstract: Impulsivity is considered a multifaceted construct that encompasses a range of behaviors including poor impulse control, premature decision-making, and the inability to delay gratification. To better understand the neural correlates of impulsivity and its facets, we performed a voxel-based lesion mapping (VLSM) analysis in a large sample of patients (N=131) with focal, penetrating traumatic brain injuries (TBI). Impulsivity was assessed using the Barratt Impulsiveness Scale (BIS-11), a 30-item self-report questionnaire, which allows for separate

measurement of global impulsivity as well as its individual facets (e.g., "motor" impulsivity). We found that heightened global impulsivity was associated with damage to multiple areas in bilateral prefrontal cortex (PFC), left insula and anterior cingulate gyrus, left temporal gyrus, and left hippocampus. Moreover, we identified a distinct cluster within left PFC (including dorsolateral PFC) associated specifically with motor impulsivity. Our results are consistent with existing literature on bilateral prefrontal cortical involvement in behavioral impulsivity, but also provide new evidence for a multidimensional representation of impulsiveness, including a left-lateralized network supporting motor impulsivity.

Disclosures: V. Mandoske: None. **A. Chau:** None. **K.K. Hauner:** None. **F. Krueger:** None. **J. Grafman:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.02/BB87

Topic: F.01. Human Cognition and Behavior

Support: DARPA Grant N66001-11-C-4006

Title: Event-related potential assessment of cognitive tasks in post traumatic stress disorder

Authors: *V. TAN, K. CORREA, A. ANKROM, C. BERKA, R. JOHNSON;
Advanced Brain Monitoring, Inc., Carlsbad, CA

Abstract: Exposure to traumatic or stressful events can lead to pathological changes within the neurobiological and psychophysiological domains that manifest, in part, as impaired cognition and abnormal reactions to everyday stimuli. Cognitive deficits such as information processing abnormalities and attention and memory dysfunction have been reported as primary symptoms in patients diagnosed with post-traumatic stress disorder (PTSD). Studies have shown that impairment can be objectively measured by using electroencephalography (EEG) to capture the event related potentials (ERPs) that are generated in response to stimuli. Previous reports revealed distinctive N2 and P3 ERP components in PTSD patients; both are commonly elicited in visual-attentional paradigms. The P3 is related to categorical and speed of information processing, and the N2 corresponds to the degree of visual attention that is being allocated to the stimulus. The present study sought to examine cognitive differences between veterans with combat-related PTSD and healthy controls using EEG and the resulting ERP measures. Thirty PTSD and thirty-six healthy comparisons were recruited for the study and were administered visually-based vigilance and memory tasks. Initial results show that the PTSD group compared to that of the healthy controls have shorter latency and attenuated amplitude of the P3 component along the central and parietal sites during the active vigilance task. In contrast, they exhibit an

overall longer latency but similar attenuation of the N2 and P3 amplitude during a recognition memory task. In addition, results show a greater amplitude for the P2 component--related to the comparing of visual input with internal representation--for the PTSD group. Overall, the results reflect evidence of lasting neuropsychological changes that occur after serious traumatic events. These changes manifest clearly in behavior and could be a valuable focal point when investigating solutions for symptomatic mitigations.

Disclosures: **V. Tan:** A. Employment/Salary (full or part-time):; Full time employee. **K. Correa:** None. **A. Ankrom:** A. Employment/Salary (full or part-time):; Part-time consultant. **C. Berka:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock holder, owner. **R. Johnson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock holder.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.03/BB88

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01DA023248

NIH Grant K02DA026990

Title: Power spectrum scale invariance as a neural marker of age, cocaine misuse and cognitive control

Authors: ***J. S. IDE**[1,2], S. HU[3], S. ZHANG[3], L. MUJICA-PARODI[2], C.-S. LI[3];
[1]UNIFESP, Sao Jose Dos Campos, Brazil; [2]Stony Brook Univ., Stony Brook, NY; [3]Yale Univ., New Haven, CT

Abstract: Background: Recent research suggests power spectrum scale invariance (PSSI) of cerebral blood oxygenation level dependent (BOLD) signals as an important neural marker of cerebral activity. In particular, because flexibility and constraint of brain circuit affect the dynamics of the hemodynamic response in a single node, PSSI can also be used to quantify circuit-wide regulation of the network. Studies by ourselves and others demonstrate that as circuits become increasingly dysregulated, signal complexity of affected nodes deviates from that critical point (Radulescu and Mujica-Parodi, 2014), as observed in trait anxiety, schizophrenia, autism, epilepsy, etc. Here we examined PSSI as a neural marker of age, cocaine misuse and cognitive control. Methods: Eighty-eight healthy (HC) and seventy-five cocaine dependent (CD) adults participated in fMRI of a stop signal task. BOLD data are preprocessed using standard methods available in SPM8, followed by additional preprocessing (de-trending,

and regressing out head movements, and white-matter and cerebrospinal fluid signals) in order to compute PSSI. The PSSI β is estimated from each FFT-transformed time series S(f) as per S(f) ∝ f^(-β) within a frequency window of 0.01-0.25 Hz. The PSSI β images are carried to second level analyses. In linear multiple-regression, we examined age related changes in PSSI each in HC and CD, and PSSI in association with years of cocaine use in CD. A critical component of cognitive control is post-signal behavioral adjustment, which is compromised in cocaine dependence. Therefore, we examined PSSI in association with post-signal slowing and compared the changes between CD and HC. Results: PSSI values are negatively correlated with age in several brain regions (fronto-parietal circuit, caudate, thalamus, as well as default-mode circuit) in the HC group, and fewer regions in the CD group. Interestingly while overall PSSI decreased with age, in several brain regions these values significantly increased with years of cocaine use (inferior frontal gyrus, putamen, parietal cortex, dorsolateral cortex). PSSI values are increased with post-signal slowing in multiple regions (ventromedial cortex, inferior frontal gyrus, dorsolateral and parietal cortex) in the HC but not in the CD groups. Conclusions: We found that PSSI is overall decreased with age, and increased with behavioral adjustment in the HC individuals. However, the same patterns are absent in the CD group, in which increased PSSI values are associated with years of cocaine use. These results highlight PSSI as a neural biomarker of cocaine misuse and its associated deficits in cognitive control.

Disclosures: J.S. Ide: None. **S. Hu:** None. **S. Zhang:** None. **L. Mujica-Parodi:** None. **C. Li:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.04/BB89

Topic: F.01. Human Cognition and Behavior

Support: Chevo Foundation

Title: Safety and feasibility of using low frequency deep brain stimulation of the subthalamic nucleus to improve cognitive performance in patients with Parkinson's disease

Authors: *K. W. SCANGOS, K. SHAHLAIE;
Dept. of Psychiatry and Behavioral Sci., Univ. of California, Davis, Sacramento, CA

Abstract: Cognitive impairment is a common feature of Parkinson's disease (PD) that results in significant disability. Executive function deficits are most frequently observed and are thought to arise from disruption in the prefrontal loop through the basal ganglia. The subthalamic nucleus (STN) is part of the indirect pathway that modulates activity through the fronto-striatal circuitry. Deep brain stimulation (DBS) of the STN is typically used to deliver continuous high frequency

stimulation to improve the motor symptoms in patients with advanced PD. It is not known if this stimulation paradigm affects cognition. In fact, there is data suggesting that high frequency stimulation of the STN impairs cognition. In contrast, some data suggests that low frequency stimulation in the theta range enhances cognitive performance. We hypothesized that theta frequency stimulation in the STN may improve executive performance in patients with Parkinson's disease. Therefore, we designed a feasibility study that would provide safety and initial-efficacy data of low frequency STN DBS on cognition. Specifically, we are testing executive task performance in 20 patients with PD at baseline and following bilateral STN DBS placement under no frequency, low frequency, and high frequency conditions. We are also examining cognitive function under a variable duration of low frequency stimulation. Executive performance is being measured through reaction time and error rates on the Stroop, N-back, and verbal fluency tasks. Safety is being monitored through the examination of psychological, cognitive, and motor consequences of low frequency stimulation. Understanding the effects of different stimulation frequencies on non-motor tasks will help elucidate the mechanism behind cognitive impairment in PD, and inform new treatment plans that optimize both motor and cognitive domains.

Disclosures: K.W. Scangos: None. **K. Shahlaie:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.05/BB90

Topic: F.01. Human Cognition and Behavior

Title: The test-retest reliability of a computerized neuropsychological cognitive test battery in chronic stroke survivors: Application for dual-task paradigms

Authors: *J. P. VORA[1], R. VARGHESE[2], T. BHATT[3];
[1]Physical Therapy, Univ. of Illinois At Chicago, chicago, ; [2]Physical Therapy, Univ. of Illinois At Chicago, chicago, IL; [3]Physical Therapy, Univ. of Illinois at Chicago, chicago, IL

Abstract: Introduction: Dual task methodologies using a secondary cognitive task are widely utilized to measure attention of the primary motor or cognitive function. While traditional paper-pencil tasks provide with valuable information regarding psychological function in an individual, they require specialized training, are prone to manual error and are not feasible to use in a dual task paradigm, limiting its overall usability particularly in clinical settings. This warrants the need for sensitive, accurate, reliable, yet affordable options of computerized testing. The purpose of this study was to establish the test- retest reliability of a computerized, custom-designed cognitive test battery in chronic stroke survivors. **Methods:** Twelve adults with chronic stroke performed the cognitive test battery that was custom-designed in and administered using

DirectRT™, Empirisoft. This battery included tasks that measured 1) visuo-motor function, via a simple and choice reaction time task (*Spot & Click, SC*); 2) associated memory, via a paired associated learning task (*Number & Position, NP*); 3) phonemic memory, via a category fluency task (*Animal Naming, AN*); 4) executive function, via the congruent and incongruent visual stroop tasks (*Color Naming, CN*); 5) discriminant decision-making, via the spatial working memory task (*Unveil the Star, US*) and 6) visual working memory, via the 1-back and 2-back tasks (*Triangle Tracking, TR*). The outcome variables consisted of reaction time in the *SC* task; accuracy for the *NP*, *CN*, *US* and *TR* tasks; total time of completion for the *US* and *CN* tasks and total number of responses in the *AN* task. The average total time to implement this battery was 25 minutes. The intraclass correlation coefficient (ICC) was used to determine test-retest reliability for all outcome variables. **Results:** There was a good to excellent test-retest reliability for all the six tasks ($p<0.05$). The ICC for *SC* task was 0.72 for simple and 0.81 for choice reaction time. Further, the ICC for accuracy on *NP* task 0.86; 0.92 for the congruent and 0.99 for the incongruent subset of the *CN* task; 0.78 for the *US* task; 0.71 for 1-back and 0.76 for 2-back for the *TR* task. The ICC for total time of completion was 0.99 for the congruent and 0.99 for the incongruent subset of the *CN* task and 0.77 for the *US* task. The ICC for the total number of responses was 0.96 for the *AN* task. **Conclusion:** Findings from this study revealed that our method of computerized cognitive testing was highly reproducible for several domains of cognitive function. Given the reliability and portability of this test, clinicians could easily implement and incorporate it in their dual-tasking assessment and intervention programs.

Disclosures: J.P. Vora: None. **R. Varghese:** None. **T. Bhatt:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.06/BB91

Topic: F.01. Human Cognition and Behavior

Title: The effects of acute psychosocial stress on higher cognitive functions: false memory formation and anchoring and adjustment

Authors: *E. PARDILLA-DELGADO[1], E. W. ASP[3], T. J. CUNNINGHAM[2], K. A. WARNER[4], J. D. PAYNE[2];
[2]Psychology, [1]Univ. of Notre Dame, South Bend, IN; [3]Psychiatry, [4]Psychology, Univ. of Iowa, Iowa City, IA

Abstract: It has been proposed that one of the functions of the prefrontal cortex (PFC), crucial for higher-order cognitive functions, is to affix ‘false tags’ to cognitive representations. Stress can negatively affect PFC activity, due to the high density of glucocorticoid receptors in this region. In this study we tested the effects of stress-related cortisol in two higher cognitive

functions: anchoring and adjustment (A&A) and false memory formation. A&A are human heuristics that are recruited when an individual needs to make a judgment or estimate a specific quantity. Individuals will use an anchor as an initial starting point, which is then adjusted until a reasonable response is reached. For example, if asked "What year was Washington elected president?" people commonly use the signing of the Declaration of Independence as an anchor. The PFC's ability to false tag helps individuals adjust away from anchors in a way that is similar to the way we correctly reject a false memory (i.e., by appropriate source monitoring). In the current study, we used a psychosocial stress task to increase stress and decrease normal PFC function. We then measured the impact of stress exposure on the executive functions of adjusting away from an anchor and source monitoring. Participants arrived in the laboratory and completed the Deese-Roediger-McDermott (DRM) false memory task and the Knowledge Estimation Task (KET) in counterbalanced order. The DRM task entailed encoding 8 lists of related words (nurse, hospital, etc.) each with an unpresented 'gist/false word', ('doctor'). After a distractor task, they completed a free recall test to assess false memory for the un-presented gist words. The KET, used to assess A&A, presented participants with questions to which they had to provide an estimation of the correct answer. For half of the questions, a true statement regarding the estimation (the anchor) was presented before the actual question. After they finished the 2 tasks, subjects were randomly assigned to a stress or control condition (n=14 per group). Following, subjects had to complete different versions of the same 2 tasks. Although data collection is still in progress, we already see that stress marginally increased recall of un-presented words compared to the control group ($t=1.91, p=.07$ for intrusions, $t=1.7$, $p=.1$ for false words). No effects were observed for recall of studied words. Thus far there are no significant differences in the KET. These preliminary results show that stress increases false memory in the DRM task, arguably by disrupting monitoring abilities, which are dependent on PFC functioning. However, we found no evidence that stress affects anchoring and adjustment.

Disclosures: E. Pardilla-Delgado: None. **E.W. Asp:** None. **T.J. Cunningham:** None. **K.A. Warner:** None. **J.D. Payne:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.07/BB92

Topic: F.01. Human Cognition and Behavior

Support: Hong Kong Research Grants Council (HKIED: GRF-841311)

Hong Kong Research Grants Council (HKIED: ECS-28403414)

Title: The role of executive function on the reading ability of Chinese adolescents: an fMRI study

Authors: *S. W. WONG[1,2], J. C. M. LO[1], H. K. F. MAK[4], K. K. H. CHUNG[3,2,1];
[1]Special Educ. and Counselling, [2]Educational and Developmental Neurosci. Unit, [3]Early Childhood Educ., The Hong Kong Inst. of Educ., Hong Kong, China; [4]Dept. of Diagnos. Radiology, The Univ. of Hong Kong, Hong Kong, China

Abstract: This study examined the role of the executive function network on the adolescent reading ability. Previous studies showed that dyslexia is associated with the deficits of cognitive abilities associated with the reading ability of adolescent students (Chung et al., 2009). However, the role of executive function on reading ability is still unclear. In this study, a counting Stroop task is used to activate the executive function network involved in resolving cognitive interference and to examine how the response of this network correlates with the reading ability of typically developing and dyslexic adolescents. The counting Stroop task is a button-press variant of the original Stroop task and is validated for examining the neural substrate of cognitive interference effect in the fMRI environment (Bush et al., 2006). **Methods:** Three typically developing male adolescents (mean age: 14) and three age-matched diagnosed Hong Kong Chinese adolescents with dyslexia completed two runs of the counting Stroop task while their brain activities were monitored with BOLD fMRI. We hypothesized that, relative to the congruent and neutral trial, the incongruent trial would elicit a stronger BOLD response within the executive function network. The reading ability of the participant is quantified with a digit rapid automatized naming (RAN) task outside the scanner. The RAN task required participants to name a series of Arabic numerals presented on a computer screen as fast as possible. The fMRI experiment was performed with the Philips 3T scanner at the MRI Unit at the University of Hong Kong. The acquired data were analyzed with SPM12. BOLD images were realigned, normalized and smoothed. A two-level statistical paradigm was used to analyze the preprocessed images. A voxel-wise generalized linear model was used to estimate the BOLD response associated with the three conditions. The RAN data were included as a covariate at the second-level model. **Results and Discussion:** The behavioral data of the counting Stroop confirmed the cognitive interference effect that the response time of the incongruent trials is significantly longer than the ones of the congruent and neutral trials. The fMRI data showed that participants' RAN performance correlated with the BOLD signal at dorsal anterior cingulate cortex (dACC) and dorsolateral prefrontal cortex (DLPFC). More specifically, participants with a higher RAN score exhibited a stronger activation at dACC and DLPFC in the incongruent trial than in the congruent or neutral trial. Our preliminary data indicated that the efficacy of dACC and DLPFC in resolving cognitive interference correlates with the reading ability of Chinese adolescents.

Disclosures: **S.W. Wong:** None. **J.C.M. Lo:** None. **H.K.F. Mak:** None. **K.K.H. Chung:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.08/BB93

Topic: F.01. Human Cognition and Behavior

Support: NMSS Grant RG4453A1

Title: Evidence for a neural-vascular coupling mechanism for individual differences in processing speed: a model-free analysis of BOLD signal in healthy and white-matter disease populations

Authors: M. P. TURNER[1], N. A. HUBBARD[1], J. L. HUTCHISON[1], H. LU[2], J. HART, Jr.[1], G. REMINGTON[3], S. L. DAVIS[4], T. FROHMAN[3], E. FROHMAN[3], *B. P. RYPMA[1];
[1]Behavioral & Brain Sci., Univ. of Texas at Dallas, Richardson, TX; [2]Radiology, Johns Hopkins Univ., Baltimore, MD; [3]Neurol., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [4]Kinesiology, Southern Methodist Univ., Dallas, TX

Abstract: Background: The hemodynamic response function (HRF), the change in brain blood flow in response to neural activity, arises from communication between neurons and the vasculature that supplies them by means of glial intermediaries known as astrocytes. In demyelinating diseases, such as Multiple Sclerosis (MS), the integrity of the white matter structure known to facilitate neural-vascular communication, of which astrocytes are an integral part, is damaged. This damage compromises the ability of neurons to adequately convey their metabolic needs, resulting in insufficient oxygen and nutrient perfusion. In this study, we isolated particular components of the HRF that could index the extent to which damaged white-matter affects neural-vascular coupling, and cognitive symptoms. Method: Fifty-one participants, 28 MS patients and 23 healthy controls, were fMRI-scanned during performance of a simple reaction time task and an fMRI-adapted version of the Digit Symbol Substitution task (DSST). A model-free approach was used to analyze the fMRI data, eliminating dependence on assumptions regarding HRF shape that may not hold for diseased populations. Several HRF metrics were calculated, including crest and trough amplitude, time-to-peak, time-to-fall, and FWHM, following the fitting of piecewise linear-B spline functions to fMRI time-series data. Results: Several metrics were significantly different, most notably crest amplitude, an index of neural activity in healthy young adults, which was attenuated in MS patients relative to controls. The extent of white matter damage, as indexed by radial diffusivity (measured by DTI), predicted the decreases seen in MS patients' HRF crest amplitudes. Further, while HRFs of healthy controls demonstrated greater variance, the incidence of non-canonical HRFs was more frequent in MS patients. Conclusions: These results support the hypothesis that dysfunction of the neural-vascular coupling system (as a result of disrupted astrocytes) leads to compromised blood flow response and cognitive impairment experienced by white-matter disease patients.

Disclosures: M.P. Turner: None. **N.A. Hubbard:** None. **J.L. Hutchison:** None. **H. Lu:** None. **J. Hart:** None. **G. Remington:** None. **S.L. Davis:** None. **T. Frohman:** None. **E. Frohman:** None. **B.P. Rypma:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.09/CC1

Topic: F.01. Human Cognition and Behavior

Support: MEXT Global Initiative for Human High Performance Research Project

Title: Acute mild exercise boosts executive performance in older adults by eliciting positive-arousal-related prefrontal activations: an fNIRS study

Authors: *K. BYUN, K. HYODO, K. SUWABE, G. OCHI, H. SOYA;
Fac. of Hlth. and Sport Sci., Univ. of Tsukuba, Tsukuba, Japan

Abstract: Executive function, which refers to higher cognitive function localized in the prefrontal cortex, decreases with aging. Although long-term mild exercise has been proven to reduce cognitive impairment in older adults, it is not known whether acute mild exercise has an enhancing effect on executive function and its neural substrates. To address this question, we examined the effect of acute mild exercise on executive function and its neural substrate using the color-word matching Stroop task (CWST), which produces a cognitive conflict in the decision-making process. Twenty-two sedentary elderly subjects (mean age: 68.8 ± 1 years; 11 females) participated in two count-balanced sessions: Exercise (Ex) and Control (Con). Subjects performed a CWST and completed a two-dimensional mood scale (TDMS) measuring psychological mood changes before and after 10 minutes of low-intensity (30% V̇O2peak) exercise on a cycle-ergometer for the Ex session, or without exercise for the Con session. During the CWST in both sessions, cortical hemodynamic changes of the prefrontal cortex were monitored using functional near-infrared spectroscopy (fNIRS). The acute mild exercise led to improved Stroop performance. It also evoked cortical activations related to Stroop interference on the left dorsal lateral prefrontal cortex and frontopolar area. Moreover, these exercise-induced cortical activations significantly corresponded with both enhanced Stroop performance and increased positive-arousal levels after exercise. These findings are consistent with our recent fNIRS study of younger adults (Byun et al., 2014), and further suggest that regardless of age, mild exercise has beneficial effects on executive function mediated by increasing task-related cortical activations.

Disclosures: **K. Byun:** None. **K. Hyodo:** None. **K. Suwabe:** None. **G. Ochi:** None. **H. Soya:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.10/CC2

Topic: F.01. Human Cognition and Behavior

Support: NIH Grants DA023248

NIH Grants DA026990

NIH Grants AA021449

Title: Ventral striatum dysfunction in cocaine dependence - a framework for resting state functional connectivity mapping analysis

Authors: *S. ZHANG, S. HU, C.-S. LI;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: Background: Recent studies started to elucidate altered resting state functional connectivity (rsFC) in individuals with cocaine dependence (CD), highlighting the effects of cocaine use on both the activities and connectivities of the ventral striatum (VS). In the current study, we developed a framework to employ rsFC and identify altered functional subclusters of the VS in cocaine dependence. **Methods**: Resting state fMRI data (3T, 10 minutes, eye closed) of 66 CD and 66 age/gender matched HC were analyzed. We performed for individual subjects linear regressions between the averaged time course of each of the 116 AAL masks and the time courses of each individual voxel of the VS. The correlation coefficients were converted to z scores by Fisher's z transform, and a two-sample t-test was applied to the "z maps" to identify altered rsFC between each VS voxel and each of the 116 masks in CD as compared to HC. We then used the 116 connectivity effect sizes (t values) of each VS voxel to functionally segment the VS with K-means clustering. **Results**: The results showed the VS being divided into 3 functional subclusters, in the area of the dorsal anterior nucleus (daVS), dorsal posterior nucleus (dpVS), and ventral nucleus (vVS), each in association with different pattern of rsFC. Besides the shared reduced rsFC with bilateral parahippocampal gyri (PHG), the three subclusters each showed increased rsFC with pre-supplementary motor area (preSMA), reduced rsFC with ventromedial prefrontal cortex (vmPFC), and increased rsFC with visual cortex in CD compared to HC, specifying the VS subregions with altered pattern of connectivity. Furthermore, the connection strengths of daVS – PHG positively correlated, while the connection strengths of vVS – PHG and dpVS – preSMA negatively correlated with the number of days of cocaine use in the past month each for females, males, and females only. **Conclusions**: These findings suggest a distinct pattern of altered rsFC of VS subnuclei in cocaine dependence. The proposed analytic framework may provide new insight to using resting state fMRI data to delineate circuit level deficits in cocaine dependence and other neuropsychiatric conditions. Supported by NIH grants DA023248, DA026990, AA021449.

Disclosures: S. Zhang: None. **S. Hu:** None. **C. Li:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.11/CC3

Topic: F.01. Human Cognition and Behavior

Support: R01-EY019882

R01-MH073028

P30-EY08126

P30-HD015052

T32-EY007135

F31-MH102042

BCS-0957072

Title: Synchronizing brain rhythms with electrical stimulation improves adaptive control in healthy people and those with schizophrenia

Authors: *R. M. REINHART, J. ZHU, S. PARK, G. F. WOODMAN;
Vanderbilt Univ., Nashville, TN

Abstract: The ability to exert control over our behavior is fundamental to human cognition. For people with psychiatric and neurological disorders impairments in adaptive control are pervasive. Schizophrenia patients in particular show deficits in reacting to errors. These enduring difficulties with executive control mechanisms that allow us to adapt have direct implications for the lives of these patients in the real world. In the present study, we report novel evidence for the neural mechanisms of adaptive control that distinguish healthy people from those with schizophrenia. We used transcranial electrical stimulation to safely, noninvasively, and casually manipulate neural activity in a targeted brain region. We combined this causal stimulation method with measurements of electrical brain activity that are hypothesized to index the large-scale neural networks underlying adaptive control. Whereas our healthy control subjects had strongly coherent low-frequency oscillations across frontal and prefrontal cortex following errors in our baseline measurements, the patients did not. However, when we passed electrical current through the medial-frontal cortex for 20 minutes, we improved adaptive control and the phase structure of patients' neural oscillations. This noninvasive stimulation phase aligned the low-frequency oscillations that were elicited after patients committed errors, requiring adaptive control to change how information is being processed. Moreover, this stimulation also causally enhanced the patterns of long-range functional connectivity carried by the theta band (4-8 Hz) across medial and lateral prefrontal cortex. This resetting of the theta-band oscillator with brain stimulation caused dramatic improvement in behavioral signatures of adaptive control, most notably the reaction time (RT) slowing observed after an error is made. Strong single-trial

correlations between theta rhythms and post-error slowing provided further behaviorally relevant insights into the neural mechanisms of adaptive control. Following stimulation the neural and behavioral indices of adaptive control were improved such that patients were indistinguishable from healthy control subjects. Theories of cortical dysconnectivity in schizophrenia are supported by our causal manipulation. But most important for translational neuroscience is the contribution our results make to the development of new, drug-free, intervention therapies for psychiatric and neurological patients with cognitive deficits.

Disclosures: **R.M. Reinhart:** None. **J. Zhu:** None. **S. Park:** None. **G.F. Woodman:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.12/CC4

Topic: F.01. Human Cognition and Behavior

Support: VA Research Service

Title: Prevalence of cognitive dysfunction apart from memory loss following traumatic brain injury from blast versus non-blast exposure in a veteran population

Authors: ***A. PAPAZYAN**[1], K. L. PANIZZON[2], J. RAMOS[3], W. STEFANOS[2], J. WATSON[2], E. A. LICHT[2], R. A. WALLIS[2,4];
[2]Neurol., [1]VA Greater Los Angeles Healthcare Syst., Los Angeles, CA; [3]Neurol., VA Greater Los Angeles, Los Angeles, CA; [4]Neurol., David Geffen Sch. of Med. UCLA, Los Angeles, CA

Abstract: OBJECTIVE: To evaluate the prevalence of cognitive dysfunction apart from memory loss after blast versus non-blast traumatic brain injury (TBI) in Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) veterans. BACKGROUND: TBI has been a signature injury in OEF/OIF veterans. After TBI, residual deficits are often seen, including cognitive dysfunction. METHODS: We conducted a retrospective chart review of patients with TBI in the Poly-Trauma Clinic of the VA Greater Los Angeles Healthcare System, regarding cognitive dysfunction in OEF/OIF veterans. RESULTS: A total of 563 charts were reviewed with 312 OEF/OIF veterans with a confirmed TBI diagnosis. The racial/ethnic distribution of subjects was 44 % Caucasian, 12% African-American, 25% Hispanic, 12% Asian and 7% other. The mean age of subjects with blast TBI was 33 ± 1 years and for non-blast TBI, 32 ± 1 years. Of 312 subjects with TBI, a mean 81 ± 6% (n = 254) were noted to have cognitive dysfunction. In subjects with post-TBI cognitive dysfunction, 57 ± 9% (n = 129) had blast-TBI, while 53 ± 9% (n = 116) had non-blast TBI. CONCLUSION: This data suggests that post-TBI cognitive dysfunction occurs often in OEF/OIF veterans.

Disclosures: **A. Papazyan:** None. **K.L. Panizzon:** None. **J. Ramos:** None. **W. Stefanos:** None. **J. Watson:** None. **E.A. Licht:** None. **R.A. Wallis:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.13/CC5

Topic: F.01. Human Cognition and Behavior

Support: NASA NNX11AR02G

NASA PF0401

NASA NCC 9-58

NASA Flight Analogs Project

National Institutes of Health

National Center for Advancing Translational Sciences, 1UL1RR029876-01

Title: Increased dual-task cost during 70-day 6° head-down bed rest (HDBR): a functional magnetic resonance imaging (fMRI) study

Authors: ***P. YUAN**[1], V. KOPPELMANS[1], P. REUTER-LORENZ[2], Y. DE DIOS[4], D. SZECSY[5], N. GADD[4], S. WOOD[6], R. RIASCOS[7], I. KOFMAN[4], J. BLOOMBERG[8], A. MULAVARA[8,9], R. SEIDLER[1,2,3];
[1]Sch. of Kinesiology, [2]Dept. of Psychology, [3]Neurosci. Program, Univ. of Michigan, Ann Arbor, MI; [4]Technol. & Engin. Group, Wyle Sci., Houston, TX; [5]Bastion Technologies, Houston, TX; [6]Dept. of Psychology, Azusa Pacific Univ., Azusa, CA; [7]The Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [8]NASA Johnson Space Ctr., Houston, TX; [9]Universities Space Res. Assn., Houston, TX

Abstract: Head-down tilt bed rest has been used as a microgravity analog to study the effects of microgravity exposure on human physiology and cognition on earth. It is reported that concurrent performance of motor and cognitive tasks can be impaired during space missions, and such dual-task costs increase during spaceflight. Effects of HDBR on brain structure have also been documented. Thus, understanding the consequences of HDBR for neural control of dual-tasking may provide insight into the cognitive effects of spaceflight. In the current study, we evaluated how dual-task costs changed as a function of HDBR. Seventeen healthy men (aged 25 to 39 years) participated in this HDBR study. They remained continuously in the 6° head-down tilt position for 70 days. Functional MRI for finger tapping was acquired during both single-task (finger tapping only) and dual-task conditions (simultaneously performing a secondary cognitive

task), and repeated at 7 time points: 12 days pre-, 8 days pre-, 7 days in-, 50 days in-, 70 days in-, 8 days post-, and 12 days post-HDBR. At all 7 time points, increased activation for finger tapping in dual-task compared to single-task condition was observed in bilateral occipital lobe and posterior parietal cortices. Additionally, increased activation in the dual-task relative to the single-task condition was significant in bilateral inferior frontal cortices and supplementary motor area during HDBR, but not before or after HDBR. Significant post-pre HDBR differences in dual-task cost were found in the left inferior parietal region. The dual-task cost of neural activity observed in the inferior frontal cortex is consistent with previous reports that the inferior frontal area is involved in the coordination of dual-task interference. Its presence during HDBR but not pre-HDBR implies that in head-down position, more neural control is needed for dual-task execution. The relationship between brain activation and behavioral performance differences in single-task and dual-task conditions will also be considered. This work was funded by the National Space Biomedical Research Institute through NASA NNX11AR02G, PF0401, NASA NCC 9-58, NASA Flight Analogs Project, National Institutes of Health, and National Center for Advancing Translational Sciences, 1UL1RR029876-01.

Disclosures: **P. Yuan:** None. **V. Koppelmans:** None. **P. Reuter-Lorenz:** None. **Y. De Dios:** None. **D. Szecsy:** None. **N. Gadd:** None. **S. Wood:** None. **R. Riascos:** None. **I. Kofman:** None. **J. Bloomberg:** None. **A. Mulavara:** None. **R. Seidler:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.14/CC6

Topic: F.01. Human Cognition and Behavior

Support: NIH AA021449

NIH DA026990

Title: Functional activities and resting state connectivity of the thalamus in non-dependent alcohol drinkers

Authors: ***S. HU**[1], S. ZHANG[1], J. IDE[2], J. KRYSTAL[1], C.-S. R. LI[1];
[1]Yale Univ., New Haven, CT; [2]Stony Brook Univ., New York, NY

Abstract: The thalamus is critical to many cognitive functions and has long been implicated in alcohol misuse. In this study, we examined the role of the thalamus in response inhibition and its resting state connectivity in association with problem drinking and duration of alcohol use. Fifty-seven non-dependent drinkers and 57 non-drinkers participated in one 40-minute fMRI scan of the stop signal task, and 44 of the drinkers also participated in a 10-minute resting state scan. Data were pre-processed and modeled using SPM8. In a GLM, we contrasted stop and go trials

with fMRI signals modeled at signal onset to identify neural correlates of response inhibition. In a group-level linear regression, we examined correlation of response inhibition with the Alcohol Use Disorders Identification Test (AUDIT) score and years of alcohol consumption. In drinkers, AUDIT score but not years of drinking was positively correlated to the stop signal reaction time. Bilateral thalamus, bilateral cerebellum, left insula, left anterior cingulate cortex (ACC), and right lateral prefrontal cortex (PFC) showed decreased activation with increasing AUDIT score. The supplementary motor area (SMA) extending to ACC and mid-cingulate cortex showed decreased activation with longer duration of alcohol use, an effect that was independent of age. In resting state functional connectivity, the thalamus as a seed region showed positive connectivity to a large array of regions including the cerebellum, ACC/SMA, dorsal medial PFC, bilateral insula, and precunues, and negative connectivity to the ventromedial PFC, precuneus, bilateral inferior parietal lobules, and bilateral middle occipital gyri. Notably, positive connectivity to the cerebellum increased with higher AUDIT score and the ACC/SMA connectivity decreased with duration of alcohol use. These results suggested altered activation of the thalamus, cerebellum, and ACC/SMA as a mechanism for deficits in response inhibition as associated with problem drinking.

Disclosures: **S. Hu:** None. **S. Zhang:** None. **J. Ide:** None. **J. Krystal:** None. **C.R. Li:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.15/CC7

Topic: F.01. Human Cognition and Behavior

Support: NIHR BRC Studentship

Title: A multimodal meta-analysis of VBM and fMRI studies in obsessive-compulsive disorder and autism spectrum disorders

Authors: ***C. O. CARLISI**[1], L. NORMAN[2], S. LUKITO[2], J. RADUA[3], D. MATAIX-COLS[4], K. RUBIA[2];
[1]Inst. of Psychiatry, King's Col. London, London, United Kingdom; [2]Child and Adolescent Psychiatry, [3]Inst. of Psychiatry, King's Col. London, London, UK, United Kingdom; [4]Dept of Clin. Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Obsessive-compulsive disorder (OCD) is characterised by intrusive thoughts (obsessions) and repetitive actions (compulsions), while autism spectrum disorders (ASD) are characterised by restricted, repetitive behaviours and interests. OCD and ASD, however, share deficits in cognitive control. However, it is unclear whether these shared behavioural deficits that contribute to their respective symptom profiles are mediated by common or distinct neural

mechanisms. The aim of the meta-analysis was to assess shared or disorder-specific neural underpinnings of inhibitory control and brain structure in these two disorders. For this purpose, we conducted a voxel-wise meta-analysis of 1) whole brain voxel-based morphometry (VBM) studies of brain structure and 2) functional magnetic resonance imaging (fMRI) studies of inhibitory control to compare regional grey matter (GM) volume and activation in OCD and ASD relative to their respective controls using Signed Differential Mapping (SDM). Multimodal analyses within each group also allowed the examination of regional differences common to both VBM and fMRI studies. Eighty-three studies across diagnostic groups and methodologies met inclusion criteria. Across fMRI studies, OCD patients (N=267) showed disorder-specific enhanced cerebellar activation while ASD (N=193) showed disorder-specific enhanced activation in lateral and ventromedial orbitofrontal cortex compared to controls. Both groups relative to controls shared underactivation in anterior cingulate cortex (ACC). In VBM, OCD patients (N=737) relative to ASD patients (N=791) had disorder-specific enhanced striato-insular and cerebellar but reduced left superior temporal and inferior frontal GM volumes. Both groups shared decreased ACC volumes relative to controls. Multimodal analyses in ASD and in OCD show that the ACC is reduced in both disorders in activation as well as GM volume. Results suggest both distinct and shared neural pathways underlying cognitive control in OCD and in ASD. Striato-insular and cerebellar regions were specifically associated with OCD and left prefrontal and superior temporal regions were specifically associated with ASD in one or both modalities, supporting evidence for disorder-specific mechanisms driving similar cognitive phenotypes of inhibitory abnormalities in each disorder. Shared mechanisms involved volumetric reduction and underactivation in ACC, presumably related to shared top-down control deficits.

Disclosures: C.O. Carlisi: None. **L. Norman:** None. **S. Lukito:** None. **J. Radua:** None. **D. Mataix-Cols:** None. **K. Rubia:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.16/CC8

Topic: F.01. Human Cognition and Behavior

Support: Pennsylvania Department of Health, Formula Grant

Title: Changes in executive function associated with tobacco cessation therapy

Authors: A. G. STIVER[1], *W. M. MEIL[1], A. SESTI[2], L. M. HAMMER[1], J. A. MILLS[1], D. J. LAPORTE[1];
[1]Psychology, [2]Alcohol, Tobacco, and Other Drugs Program, Indiana Univ. Pennsylvania, Indiana, PA

Abstract: This study examined changes in functions associated with the prefrontal cortex as a result of tobacco cessation treatment in college students. Fourteen participants seeking tobacco cessation treatment from a university based tobacco cessation program and seventeen control participants were tested with the battery of neuropsychological measures prior to treatment and again 1 ½ months later. Participants were administered the Delis-Kaplan Executive Function System (D-KEFS) test battery and completed questionnaires including the Sensation Seeking Scale V (SSS-V), the Frontal Systems Behavioral Scale (FrSBe), the Perceived Stress Scale (PSS), the Fagerstrom Test of Nicotine Dependence (FTND), and answered questions regarding their frequency of tobacco use. Tobacco cessation treatment included the option of receiving the Nicotine Replacement Patch and Motivational Interviewing Therapy. Tobacco cessation treatment participants attended an average of 4.5 (SD = 2.34) meetings with treatment personnel. They were provided an average of 3 (SD =1.35) boxes of nicotine patches. The mean number of days between the first and last treatment meeting was 35.85 (SD = 17.74).There was a significant decrease in FTND scores between the first (M = 3.38, SD = 2.57) and second testing session (M = .923, SD =.93; $t(12) = 3.39$, $p = .004$). A Mixed Model Analysis of Variance was used to compare treatment condition and test time on SSSV, PSS, FrSBe subscores, and D-KEFS composite scores. Significant Interactions were observed between treatment condition and test time on SSSV ($F(1,27) = 5.14$, $p = .032$, partial $\eta 2 = .160$), FrSBe Apathy subscores ($F(1,28) = 6.40$, $p = .017$, partial $\eta 2 = .186$), FrSBe Disinhibition subscores ($F(1,28) = 4.48$, $p = .043$, partial $\eta 2 = .138$), and FrSBe Executive Function subscores ($F(1,28) = 7.07$, $p = .013$, partial $\eta 2 = .202$). Tobacco cessation participants showed decreases across test times while control subjects scores tended to increase. Difference scores on the FrSBe Executive Function subscale were significantly correlated with the number of nicotine patches participants were provided ($r = .81$, $p = .001$), the number of days between the first and last treatment session ($r = .58$, $p = .029$), and approached significance for the number of meetings with treatment personnel ($r = .53$, $p = .053$). These results suggest tobacco cessation therapy is associated with short-term improvements in executive function particularly when using ecologically based self-report measures of frontal lobe function.

Disclosures: **A.G. Stiver:** None. **W.M. Meil:** None. **A. Sesti:** None. **L.M. Hammer:** None. **J.A. Mills:** None. **D.J. LaPorte:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.17/CC9

Topic: F.01. Human Cognition and Behavior

Support: NIH DA023248

NIH DA026990

NIH AA021449

Title: Resting state functional connectivity of the ventral striatum: hemispheric laterality, gender difference and cocaine addiction

Authors: *C.-S. R. LI, S. ZHANG, S. HU;
Dept Psychiatry, Yale Univ., New Haven, CT

Abstract: BackgroundResting state functional connectivity (rsFC) is widely used to examine cerebral functions. The rsFC of the ventral striatum (VS) has been implicated in motivated behaviors and clinical conditions that compromise motivated behaviors. There is a literature that suggests functional differences between left- and right- hemispheric VS. The current study investigated this issue and explored the effects of gender and cocaine addiction. MethodsWe used flexible factorial analyses to examine whole-brain rsFC and left and right VS and the effects of gender in a sample of 250 young healthy adults and examine the effects of cocaine addiction on left and right VS connectivity in a cohort of 66 cocaine-dependent (CD) and 66 age- and gender- matched healthy individuals (HC). Data preprocessing and computation of individuals' correlation maps followed our previous work (Zhang et al., 2012 Cereb Cortex). We also employed the AAL masks in ROI analysis to further confirm the findings. ResultsFirst, left- and right- VS each showed greater rsFC with regions within the same hemisphere with women showing qualitatively greater laterality. With both VS combined, men showed greater rsFC to the SMA, dACC, thalamus, insula, midbrain, while women showed greater rsFC to the visual cortex, OFC, caudate head, temporal pole, and cerebellum, at $p<0.05$ FWE. Compared to HC, CD showed greater VS rsFC to bilateral OFC, lateral PFC, visual cortex, and caudate head and less rsFC to parahippocampal gyri (PHG), inferior temporal cortex, temporal pole, vmPFC, posterior cingulate cortex/precuneus. There were no notable differences between CD and HC in the laterality effects. Furthermore, the connection strengths of VS - PHG and VS - lateral PFC negatively correlated with the average amount of cocaine per use in the past month each for males and for females only. And the connection strengths of VS - vmPFC positively correlated with the cocaine abstinence days for males but not for females. ConclusionsThese findings highlight significant effects of gender and cocaine addiction in VS rsFC and along with a broader literature suggest the importance of considering gender in studies of VS functioning in cocaine addiction and many other clinical conditions.

Disclosures: C.R. Li: None. **S. Zhang:** None. **S. Hu:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.18/CC10

Topic: F.01. Human Cognition and Behavior

Support: NSERC

Title: The influence of a single session of aerobic exercise on error processing and subsequent executive control during a flanker task

Authors: *K. B. BEYER[1], M. D. SAGE[2], W. E. MCILROY[2];
[2]Kinesiology, [1]Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Aerobic exercise may positively influence response conflict and error monitoring processes that engage executive control to improve future behaviour. After a behavioural task trial containing a response error (inherently high in response conflict), the performer will slow down on the next trial to ensure a correct response. Furthermore, cortical activity related to processing a response error is predictive of changes in behaviour (e.g., speed and accuracy) and cortical activity related to executive control on subsequent trials. Aerobic fitness and long-term aerobic training are associated with changes in behaviour following an error as well as cortical activity related to both error processing and executive control. Even a single session of aerobic exercise can influence cortical activity related to executive control, but it is unclear how cortical activity related to error processing following a response error and subsequent behaviour are influenced. We used behavioural and electrophysiological measures to probe the relationship between response errors, subsequent behaviour, and associated cortical activity and examined how this relationship was influenced by a single session of aerobic exercise. Participants performed a modified flanker task before and after a 40-minute session of moderate-intensity cycle ergometry. For each flanker trial, reaction time was the time elapsed from stimulus presentation to EMG onset. Response accuracy was the percentage of trials with the correct response. Electroencephalography was used to index cortical activity related to error processing and executive control (e.g., ERN and P3 ERP components). On incongruent flanker trials immediately following an error, participants reacted slower and were more accurate than after a correct response indicating an error-related increase in executive control on subsequent trials. After aerobic exercise, participants reacted even slower following error trials indicating a further enhancement to executive control in response to errors. These findings indicate that, like aerobic fitness and long-term aerobic training, a single session of aerobic exercise can influence executive control of behaviour following a response error. Ongoing analysis is examining the relationships between cortical activity associated with error processing, cortical activity associated with executive control, and the observed changes in behavioural performance on trials following a response error.

Disclosures: **K.B. Beyer:** None. **M.D. Sage:** None. **W.E. McIlroy:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.19/CC11

Topic: F.01. Human Cognition and Behavior

Title: Effect of an acute bout of soccer heading on executive function in soccer players

Authors: *C. WALLACE[1], K. BRYK[2], A. D. WRIGHT[1], M. KENNEFICK[1], P. VAN DONKELAAR[1];
[1]Human Kinetics, [2]UBC Okanagan, Kelowna, BC, Canada

Abstract: Concussion often causes executive function deficits, which can be measured using a simple task-switching paradigm. However, little is known about subconcussive impacts and their influence on executive function. This particular study examined executive function in college-aged soccer players immediately prior to and following an acute bout of soccer heading, with the participant heading a soccer ball fired from a Sports Tutor soccer machine 20 times in 5 minutes, along with a sham condition where participants used their chest to direct the ball. Switch costs for both conditions showed no statistical difference. We conclude that an acute bout of soccer heading has no immediate effect on executive function when compared to a sham condition.

Disclosures: C. Wallace: None. **K. Bryk:** None. **A.D. Wright:** None. **M. Kennefick:** None. **P. van Donkelaar:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.20/CC12

Topic: F.01. Human Cognition and Behavior

Title: Effect of yoga practice on cognitive-motor interference of dynamic balance control

Authors: *S. SUBRAMANIAM, S. NADIMPALLI, T. BHATT;
Rehabil. Sci., 2680 Dunrobin Cirlce,, Chicago, IL

Abstract: ***Background***: It is postulated that balance centers within the central nervous system share attentional resources with the centers for cognitive processing. This results in deterioration of either one or both tasks under dual-task (DT) conditions, known as cognitive-motor interference (CMI). Apart from the beneficial effects on balance control, yoga positively influences several aspects of cognition, especially executive function. In spite of its motor and cognitive benefits, the effect of yoga practice on DT performance during varied balance tasks has not been examined. ***Purpose***: The purpose of our study was to investigate the effects of yoga on reducing CMI for maintaining dynamic balance during varied balance tasks. ***Method***: Yoga

practitioners (N=10) and age-similar non-practitioners performed three balance tasks including the Limits of Stability test (LOS) (intentional balance), Motor Control test (MCT) (reactive balance), and Sensory Organization Test (SOT) (condition 6 - inducing somatosensory and visual conflicts) under single-task (ST) and dual-task (DT) conditions (in conjunction with a serial subtraction task). The motor performance was assessed by recording the response time (RT) and movement velocity (MV) of the center of pressure (CoP) on LOS task, weight symmetry (WS) of CoP on the MCT task and equilibrium (EQ) of CoP on the SOT task. Cognitive performance was assessed by recording the number of correct responses in sitting (ST) and the DT conditions. The motor cost (MC) and cognitive cost (CC) was computed using the formula ([ST-DT]/ST)*100 for the motor and cognitive variables. Greater cost values indicate lower performance under DT versus ST conditions. ***Results***: The yoga group showed a significantly lesser motor cost for the spatial variables on the LOS, MCT and SOT tests (MV, WS, EQ respectively, $p<0.05$) in comparison with the control group. For the RT, the yoga group compared with the control group had a significantly lesser cost for the LOS task (intentional balance) but not for the MCT task (reactive balance). Similarly, the cognitive costs were significantly lower for all the three balance tasks for the yoga group than the control group ($p<0.05$). ***Conclusion***: Our results suggest that yoga practice can significantly reduce CMI by improving allocation and utilization of attentional resources for both dynamic balance control and cognitive functioning; hence resulting in better performance under DT conditions. Yoga practice could thus reduce fall risk by improving balance control under challenging environmental conditions that may require simultaneous processing of information from multiple sources.

Disclosures: S. Subramaniam: None. **S. Nadimpalli:** None. **T. Bhatt:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.21/CC13

Topic: F.01. Human Cognition and Behavior

Support: NDSEG

NIH Grant MH63901

NIH Grant NS79698

NIH Grant NS090757

Title: Influence of selective attention on brain network reconfiguration during working memory

Authors: *C. L. GALLEN[1], K. HWANG[1], T. G. LEE[2], M. D'ESPOSITO[1];
[1]Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA; [2]Dept. of Psychological and Brain Sci., UC Santa Barbara, Santa Barbara, CA

Abstract: Selective attention underlies our ability to attend relevant stimuli while ignoring irrelevant stimuli, a critical process for prioritizing information in working memory. Recent evidence suggests that brain networks reconfigure during working memory performance, such that there is more integration across network modules (i.e., less modular) with increased working memory load. However, it is not yet understood how brain networks reconfigure between the processing of relevant and irrelevant stimuli in the service of working memory. Here, we investigated the effects of selective attention on network structure during working memory performance. We created networks from fMRI data collected during a modified one-back working memory task for 38 subjects. In each trial, subjects viewed a series of faces and scenes. Attention to the stimuli was manipulated in three conditions. Subjects were instructed to (1) attend faces and ignore scenes, (2) attend scenes and ignore faces, or (3) categorize the image as a face or scene. We used the first two conditions to examine processing of relevant (i.e., attended) and irrelevant (i.e., unattended) stimuli during working memory performance. The third condition served as a comparison in which no working memory was required. We created networks for each condition and three metrics were used to assess changes in modular network organization due to attention demands. We first examined modularity, a comparison of within- to between-module connections. We also directly quantified the number of between-module network connections. Finally, we identified nodes that had many within-module connections in the categorize condition and assessed how these changed due to selective attention. Participants had reduced modularity and increased between-module connections for relevant stimuli compared to irrelevant and categorize conditions. Further, nodes with many within-module connections in the categorize condition decreased their within-module connectivity during the relevant condition. We next investigated how network reconfiguration due to attention demands is related to task performance, by grouping subjects into fast and slow performers for relevant stimuli. Fast subjects showed greater decreases in modularity and increases in between-module connections to relevant compared to irrelevant stimuli. These results suggest that networks rapidly reconfigure while attending relevant stimuli to adopt a more globally distributed organization in which there is greater communication between modules. Further, more integration across network modules to attended stimuli supports better working memory performance.

Disclosures: **C.L. Gallen:** None. **K. Hwang:** None. **T.G. Lee:** None. **M. D'Esposito:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.22/CC14

Topic: F.01. Human Cognition and Behavior

Support: Parkinson Study Group and the Parkinson's Disease Foundation

NIH Grant DA034685

Title: Dopamine-dependent functional network reorganization in Parkinson's disease and its relationship to working memory

Authors: *R. L. WHITE[1,2], M. D'ESPOSITO[2];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Parkinson's disease (PD) is often accompanied by impairment in executive function and working memory (WM), which may be related to the dopamine depletion that occurs in PD. Exogenous dopamine can either enhance or disrupt WM in PD patients, depending on baseline characteristics. While recent evidence suggests that global integration of brain networks supports WM in healthy individuals, it is not clear how network properties are modulated in PD or how this affects WM function. We hypothesized that brain network organization would be altered in PD patients, and would be ameliorated after administration of dopaminergic medication. Furthermore, we hypothesized that the magnitude of network reorganization would capture changes in WM performance. We created network graphs from fMRI data collected during a resting-state scan in 24 PD patients and 20 matched control subjects. Each patient was scanned both ON and OFF their usual dopaminergic medication on separate days, with order counterbalanced across subjects. Performance of a WM task with distraction was measured immediately following the scan. Metrics derived from network graphs allowed us to assess functional network properties that were altered in PD and modified by exogenous dopamine. Graphs were generated from functional connectivity matrices between 333 cortical atlas nodes and were then partitioned into communities. Modularity was used to quantify the clustering of nodes into communities. The participation coefficient (PC) of nodes was used to quantify the proportion of connections between communities. We found that modularity was reduced in patients OFF medication and was normalized ON medication. WM performance (accuracy and reaction time) was impaired OFF medication and also improved ON medication. However, drug-induced changes in modularity were not directly correlated with changes in WM performance. Instead, dopamine-induced changes in WM performance correlated with more dopamine-induced PC of nodes in the default and somatomotor networks, indicating that these sub-networks increased their coupling with other sub-networks in support of WM. These results provide evidence that dopamine loss in PD is associated with a more modular, less distributed brain network organization at rest, and dopamine-dependent changes in the connectivity between sub-networks appear to play an important role in PD-related WM dysfunction. These results suggest that dopamine-induced changes in functional brain organization may be an important factor in determining the effects of dopamine on behavior.

Disclosures: R.L. White: None. **M. D'Esposito:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.23/CC15

Topic: F.01. Human Cognition and Behavior

Support: NSF IGERT Grant 0903622 to PLB

Beckman Institute Graduate Fellowship to PLB

NDSEG to CLG

ONR Grant N000140710903 to AFK

Title: Brain network predictors of cognitive training-related gains in young adults

Authors: *P. L. BANIQUED[1], C. L. GALLEN[1], M. B. KRANZ[2], A. F. KRAMER[2], M. D'ESPOSITO[1];
[1]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; [2]Beckman Inst. for Advanced Sci. and Technology, Dept. of Psychology, Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The brain operates via networked activity in separable groups of regions called modules. Modularity is quantified as the relative number of connections between modules to connections to within modules, and thus characterizes the balance of integration and segregation in a network. We have previously demonstrated that higher baseline brain modularity can predict response to cognitive training in patients with traumatic brain injury (TBI). In healthy adults, however, the functional significance of modularity in predicting training outcomes is not known. Here we examined brain modularity in young adults aged 18-30 who underwent cognitive training with working memory and reasoning (WM-REAS) casual video games. At post-training, WM-REAS participants improved in divided attention measures relative to an active control game group and a no-contact control group. In the WM-REAS groups (N=81), we derived brain networks using data obtained from a six-minute resting state fMRI scan administered prior to training. Modularity was computed from functional connectivity matrices generated using 264 predefined nodes. Similar to TBI patients, modularity measured at baseline was positively correlated with improvement in divided attention following training. Baseline cognitive ability as assessed by six tests of fluid intelligence (Gf) was negatively related to divided attention gain, and highlights the role of individual differences in predicting training improvement. We performed a median split based on individuals' pre-training Gf scores and found that baseline modularity predicted training-related divided attention gain in low Gf, but not high Gf participants. In the low Gf group, individuals with higher baseline modularity showed greater improvements in divided attention, even after controlling for baseline Gf. These results are

consistent with the idea that a more modular brain may allow for greater adaptive re-organization during cognitive training. On a broader scale, these findings suggest that, in low-performing individuals, global network properties can capture unique aspects of brain function that are important in understanding individual differences in learning.

Disclosures: P.L. Baniqued: None. **C.L. Gallen:** None. **M.B. Kranz:** None. **A.F. Kramer:** None. **M. D'Esposito:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.24/CC16

Topic: F.01. Human Cognition and Behavior

Title: Decreasing the amplitude of contingent negative variation through long-term consecutive visual search

Authors: *K. OKI[1], R. KOSHIZAWA[2], M. TAKAYOSE[3];
[1]Nihon Univ., Funabashi, Chiba, Japan; [2]Col. of Commerce, Nihon Univ., Setagaya, Tokyo, Japan; [3]Nihon Univ., Narashino, Chiba, Japan

Abstract: We investigated the influence of a long-term consecutive visual search on the contingent negative variation (CNV). Ten subjects, who were all male, performed a choice reaction task (CRT) before and after a long-term consecutive visual search task. In CRT, the up- or down-pointing arrow was shown at the center of the display as the warning stimulus (S1), and a square target as the imperative stimulus (S2) subsequently appeared upside or downside of the central cue. When the direction of S1 and S2 corresponded (this is called the target stimulus), each subject, conducting 80 trials, was supposed to respond with his right thumb pressing the joystick button. As for the long-term consecutive visual search task, the Advanced Trail Making Test Random Task (ATMT Task R) was introduced, in which a subject, using a computer mouse, clicked a black circle with the numbers from 11 to 40 in serial order. Once a numbered target was clicked, it was to disappear and another circle with the clicked number plus 30 showed up at the same time, all the circles rearranged at random; 30 circles were on the screen at any time. Each subject performed the task 40 times. During these trials, the subjects' EEG was recorded from Cz electrode along with the international 10-20 system. The averaged EEG patterns of each subject, were utilized in our analysis. The mean amplitudes of the early CNV components (550-750 ms after S1) and the late CNV components (1800-2000 ms after S1) were calculated, and the values before and after the long-term consecutive visual search were compared. It was clarified that the amplitudes of both the early and the late CNV components significantly decreased after the search, compared with those before the search. As the early CNV components reflected the orienting attention to S1 and, on the other hand, the late ones were closely related to the

attention, anticipation and response preparation to S2, the long-term visual search impaired the orientation to S1 and the three factors to S2.

Disclosures: **K. Oki:** None. **R. Koshizawa:** None. **M. Takayose:** None.

Poster

354. Human Executive Function: Clinical and Translational

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 354.25/CC17

Topic: F.01. Human Cognition and Behavior

Support: JSPS Grant-in-Aid for Young Scientists (B) #25870790

Title: The relationship between prefrontal cortex activity during preparatory period and inhibition task performance

Authors: ***M. TAKAYOSE**[1], R. KOSHIZAWA[2], K. OKI[3];
[1]Nihon Univ. Col. of Industrial Technol., Chiba, Japan; [2]Nihon Univ. Col. of Commerce, Tokyo, Japan; [3]Nihon Univ. Col. of Sci. and Technol., Chiba, Japan

Abstract: The prefrontal cortex (PFC) plays a critical role in response inhibition. PFC activity during the preparatory period affects task performance, but the mechanism by which PFC activity contributes to accurate response inhibition remains unclear. To elucidate this mechanism, contingent negative variation (CNV) was compared in different task performances. In the present study, CNV during a modified stop-signal task was examined in 10 healthy right-handed participants. Participants were required to press a button when a go signal was presented but to withhold the response when the go signal was followed by a stop signal. EEG data were recorded from the scalp using 128 channels. Six channels (Fz, F3, F4, Cz, C3, and C4), in accordance with the international 10—20 system, were adopted as regions of interest. Surface electromyograms (EMGs) were recorded from the abductor pollicis brevis. Task performances were separated into unsuccessful inhibitions (UI), successful inhibitions with EMGs (SI-EMG), and successful inhibitions without EMGs (SI-noEMG). The preparatory period of each performance was compared and analyzed. Early CNV amplitudes of F4 and C4 were larger with UI than that with the other performances. Late CNV amplitudes of all channels, with the exception of C4, were smaller with SI-EMG than that with the other performances. These findings suggest that the contribution of PFC activity to response inhibition differs according to the components of CNV. Late CNV likely facilitates the inhibition process after the presented go signal, whereas early CNV facilitates the execution process.

Disclosures: **M. Takayose:** None. **R. Koshizawa:** None. **K. Oki:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.01/CC18

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant MH078064 to JR

Title: Hippocampal and cortical M1 and M3 in cognitive and affective behaviors

Authors: *K. LEADERBRAND[1], H. J. CHEN[1], K. A. CORCORAN[1], S. TONEGAWA[2], J. WESS[3], J. RADULOVIC[1];
[1]Northwestern Univ., Chicago, IL; [2]MIT, Cambridge, MA; [3]Natl. Inst. of Diabetes and Digestive and Kidney Dis., Bethesda, MD

Abstract: The hippocampus (HPC) and retrosplenial cortex (RSC) are inter-connected structures that play prominent roles in learning and memory processes, and show functional and structural alterations in psychiatric illness. For example, damage to either region can result in amnesia, and deficits in RSC and HPC connectivity are associated with serious mental illnesses, such as depression and schizophrenia. Both the RSC and HPC are heavily innervated by cholinergic projections from the medial septum and diagonal band of Broca. In addition, the cholinergic system has been strongly implicated in memory, as well as anxiety- and depression-like behavior. We therefore investigated the role of cholinergic neurotransmission in both of these regions in mouse models of fear-related learning and memory, and affective behaviors, including contextual fear conditioning, novel object recognition, novelty-suppressed feeding, and the forced swim test. Utilizing both pharmacological and genetic techniques, we demonstrated the specific roles of the M1 and M3 muscarinic receptor subtypes in each region for these behaviors, and examined their potential downstream signaling pathways. Identifying these mediators of cognitive and affective behavior is crucially important for developing a complete model of and potential therapies for memory and mood disorders.

Disclosures: K. Leaderbrand: None. **H.J. Chen:** None. **K.A. Corcoran:** None. **S. Tonegawa:** None. **J. Wess:** None. **J. Radulovic:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.02/CC19

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant R01-MH078064 to JR

Dunbar Funds to JR

Title: Altered states: amnestic treatments alter coherent activity between retrosplenial cortex and associated structures during memory retrieval

Authors: *K. A. CORCORAN[1], B. J. FRICK[1], L. M. KAY[2], J. RADULOVIC[1];
[1]Psychiatry & Behavioral Sci., Northwestern Univ., Chicago, IL; [2]Dept. of Psychology, Inst. for Mind and Biol., Univ. of Chicago, Chicago, IL

Abstract: Activity in retrosplenial cortex (RSC) is often reflected in human disorders characterized by aberrant memory processing: it is one of the earliest brain regions to show metabolic decline in Alzheimer's disease, and increased activation of RSC is consistently seen during retrieval of traumatic memories in PTSD patients. Memory retrieval requires both activity in RSC and is associated with changes in coherent activity between RSC and connected structures. Many disorder-related memory deficits can be modeled using amnestic drugs that target specific neurotransmitter systems, but how these drugs affect inter-regional activity within memory networks is unknown. We therefore explored the patterns of RSC oscillatory activity underlying retrieval of fear-evoking memories, and during retrieval failure after amnestic treatment. Mice were trained to fear a context paired with footshock, and then tested for memory retrieval beginning the following day. Retrieval tests were performed after systemic injection of the muscarinic cholinergic receptor antagonist scopolamine, the NMDA glutamate receptor antagonist MK-801, or the extrasynaptic GABAa receptor agonist gaboxadol. Using a wireless NeuroLogger system (TSE Systems), we obtained simultaneous recordings of continuous local field potential (LFP) activity during memory retrieval from RSC as well as hippocampus, lateral septum, and anterior cingulate cortex (ACC), regions connected to RSC that have also been implicated in fear memory. We measured coherence of activity between these regions through a range of frequencies along with changes in coherence during memory retrieval both on and off drugs. Identifying specific LFP patterns has the potential to unravel subtle mechanisms of circuit interactions associated with memory retrieval and how amnestic agents function to disrupt memory retrieval at a network level.

Disclosures: **K.A. Corcoran:** None. **B.J. Frick:** None. **L.M. Kay:** None. **J. Radulovic:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.03/CC20

Topic: F.02. Animal Cognition and Behavior

Support: grants from the National Research Foundation (2013R1A1A3A04-006580) and the Oromaxillofacial Dysfunction Research Center for the Elderly at Seoul National University in Korea (2014050477) funded by the Ministry of Science, ICT and future Planning

Title: Nitric oxide in the extinction memory formation of lithium-induced conditioned taste aversion learning

Authors: *J. JAHNG[1], V. RYU[2], J. Y. KIM[1], J. .-H. LEE[1];
[1]Seoul Natl. Univ. Sch. Dent., Seoul, Korea, Republic of; [2]Dept. of Biol., Georgia State Univ., Atlanta, GA

Abstract: Lithium chloride is conventionally used as an unconditioned stimulus (US) in the formation of conditioned taste aversion (CTA), a form of classical conditionings. Lithium chloride increases both the synthesis and activity of nitric oxide synthase in the brain regions implicated in CTA learning, and nitric oxide donors have been reported to produce a CTA in rats. This study was conducted to examine tentative implications of nitric oxide and nicotinic receptor activation in the acquisition and extinction of lithium-induced CTA learning. Rats were pretreated with nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) or nicotinic acetylcholine receptor antagonist mecamylamine either at the conditioning (sucrose-lithium pairing) or at each drinking test. L-NAME prior to lithium chloride (US) did not affect the lithium-induced CTA formation; however, L-NAME at a dose of 30 mg/kg prior to each sucrose (conditioned stimulus; CS) drinking test significantly suppressed CS intake. Mecamylamine prior to US did not affect the acquisition of lithium-induced CTA, but at high dose (2 mg/kg) it facilitated the extinction. Mecamylamine (2 mg/kg) prior to each CS test delayed the extinction of lithium-induced CTA memory. Results suggest that nitric oxide may be implicated in the extinction memory formation of lithium-induced CTA, possibly in relation with the activation of nicotinic acetylcholine receptor.

Disclosures: J. Jahng: None. **V. Ryu:** None. **J.Y. Kim:** None. **J.-. Lee:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.04/CC21

Topic: F.02. Animal Cognition and Behavior

Support: NINDS Grant P01NS045260-01

NINDS Grant R01NS057128

Title: PHLPP1 knockout promotes spine actin polymerization, long-term potentiation, and learning in male but not female calpain-1 knockout mice

Authors: *Y. LIU, Y. WANG, J. SUN, D. LOPEZ, X. BI, M. BAUDRY;
Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Our previous studies have shown that long-term potentiation (LTP) elicited by theta burst stimulation (TBS) as well as learning of hippocampus-dependent tasks are impaired in calpain-1 knock-out (KO) mice. Calpain-mediated truncation of the Leucine rich repeat Protein Phosphatase 1 (PHLPP1) and the resulting stimulation of local protein synthesis play an important role in LTP induction and consolidation. To further evaluate the role of PHLPP1 in calpain-related signal pathways, we generated calpain-1-/- and PHLPP1-/- (DKO) and their respective controls, calpain-1-/- and PHLPP1+/+ (C1KO) mice. As previously reported, TBS produced a normal enhancement of field EPSPs in hippocampal slices prepared from C1KO mice but EPSPs returned to baseline by 30 min after TBS. In contrast, LTP in slices from male but not female DKO mice stabilized at levels found in wild-type controls. TBS-induced actin polymerization within dendritic spines, an essential event for stabilizing LTP, was absent in slices from C1KO mice but was clearly present in those from male but not female DKO mice. Long-term memory scores in a fear-conditioning paradigm were reduced by 30% in C1KO mice but were comparable to values found in wild-type control and in male but not female DKO mice. Consistent with these findings, C1KO mice displayed significant deficits in novel object recognition but wild-type control mice and male but not female DKO mice. Together, these data support the critical role of calpain-1-mediated cleavage of PHLPP1 in LTP induction. Deletion of both calpain-1 and PHLPP1 possibly trigger LTP and the resulting actin polymerization and learning of hippocampal tasks by activating a different signaling pathway. Further studies are needed to account for the dramatic sex differences in the DKO mice.

Disclosures: Y. Liu: None. **Y. Wang:** None. **J. Sun:** None. **D. Lopez:** None. **X. Bi:** None. **M. Baudry:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.05/CC22

Topic: F.02. Animal Cognition and Behavior

Title: Modelling a relationship between hippocampal theta frequency and long-term potentiation in anxiety tests

Authors: *G. CASTEGNETTI[1], D. BUSH[2], D. R. BACH[1,3];
[1]Dept. of Psychiatry, Psychotherapy and Psychosomatics, Univ. of Zurich, Zurich, Switzerland; [2]Inst. of Cognitive Neurosci., [3]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstract: Rodent approach/avoidance conflict tests such as the elevated plus maze or the open field test elicit anxiety-like behaviours which are reduced by anxiolytic drugs. Compared to familiar environments, these tests also increase the power of theta oscillations (4-8Hz) of the hippocampal local field potential (LFP). The frequency of these oscillations is slightly but consistently reduced by anxiolytic drugs. Beyond anxiety models, theta oscillations are known to appear during translational movement and arousal, to modulate the firing rate and spike timing of principal cells in the hippocampal formation and amygdala, and to be important for spatial memory function. However, their functional relation to anxiety-like behaviours is currently unclear. Synaptic plasticity is crucial for encoding threat memories through potentiation of amygdala inputs. Here, we simulate the impact of perturbations of theta frequency on long-term potentiation. We model two integrate-and-fire neurons connected by a single synapse, the weight of which is governed by a calcium dependent synaptic plasticity rule. These neurons receive constant feed-forward excitation and theta rhythmic inhibition which causes them to present a periodic behaviour of the membrane potential. Our simulations demonstrate that the frequency of the theta oscillation affects synaptic plasticity in a scenario where the presynaptic neuron fires once in each theta cycle. Under such conditions, theta frequency has a positive, monotonic relationship with increases in synaptic strength during learning. This modulatory effect is more pronounced when the postsynaptic neuron is weakly excited. In this case, the slope of the predicted relationship between theta frequency and synaptic strength supports the possibility that frequency variations as small as those typically elicited by anxiolytics might suffice to significantly reduce long-term potentiation. Our results suggest that theta frequency changes evoked by anxiolytic drugs might be related to an altered encoding of threat predictions, possibly explaining changes in observed anxiety-like behaviours.

Disclosures: **G. Castegnetti:** None. **D. Bush:** None. **D.R. Bach:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.06/CC23

Topic: F.02. Animal Cognition and Behavior

Support: MEXT Brain/MINDS project

JSPS KAKENHI 15H04265

CREST JST

Title: Habenular spike-tiggered analysis of spike firing in hippocampal neurons

Authors: *N. SHAFEGHAT[1], H. AIZAWA[2], H. OKAMOTO[1], T. FUKAI[1];
[1]Riken, Saitama Prefecture, Japan; [2]Tokyo Med. and Dent. Univ., Med. Res. Institute, Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: The lateral habenula (LHb) has drawn growing attention as this region could contribute to the regulation of dopaminergic and serotonergic activities in the central nervous system. We previously showed that LHb activity is crucial for the maintenance of hippocampal theta oscillation and that the majority of hippocampal neurons exhibit phase-locked activity to the oscillation. These results and the well-known theta phase precision of hippocampal neuronal firing during spatial navigation motivated us to investigate whether the hippocampus displays spike sequences time-locked to firing of LHb neurons. To this end, we analyzed the data of simultaneous multiunit recordings of LHb and hippocampal neuron ensembles in sleeping and behaving rats. Our inhouse spike sorting algorithm (EToS4) yielded sufficiently many units for this analysis. Cross-correlation analysis revealed sharp peaks at various timings within theta cycle time window in the cross-crrelograms between a LHb neuron and multiple hippocampal neurons. The results indicate that triggered by LHb activity, some hippocampal neurons fire in a specific temporal order of millisecond timescale. Such sequences may have implications in positive and/or negative reward-driven processing of memory.

Disclosures: **N. Shafeghat:** None. **H. Aizawa:** None. **H. Okamoto:** None. **T. Fukai:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.07/CC24

Topic: E.05. Stress and the Brain

Support: Indiana University

Title: Dendritic reorganization in medial prefrontal cortex of male and female rats following recovery from chronic stress

Authors: *K. M. MOENCH, C. L. WELLMAN;
Dept. of Psychological and Brain Science, Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Chronic stress produces differential dendritic remodeling of pyramidal neurons in medial prefrontal cortex of male and female rats (Garrett & Wellman, 2009). In males, the dendritic remodeling is reversible, with dendritic lengths returning to unstressed levels by 21 days after the cessation of stress (Radley et al, 2005). However, the timeline of this recovery, as well as the potential for reversibility in females, has yet to be established. Here, we examined dendritic recovery of pyramidal neurons in prelimbic cortex of male and female rats following

chronic restraint stress (3 h/day for 10 days). Following chronic stress, half of the rats were perfused immediately, and half of the rats were perfused following a 10 day recovery period. This time point was chosen due to previous work demonstrating this to be a sufficient amount of time for recovery of hippocampal pyramidal neurons following chronic stress (Conrad et al., 1999). Rats were overdosed and brains were stained using a Golgi-Cox procedure. Pyramidal neurons in layer II-III of prelimbic cortex were drawn in three dimensions, and the morphology of apical and basilar arbors was quantified. As expected, chronic stress produced apical dendritic retraction prelimbic cortex in males. Further, after recovery, morphology of neurons from stressed rats resembled that of unstressed animals. Dendritic retraction was also seen in female rats. However, the magnitude of this retraction was reduced compared to that seen in male rats. Additionally, hemispheric differences were found in the pattern of apical retraction in females but not males, with greater retraction in the left hemisphere. Similar to males, dendritic retraction in females was ameliorated by 10 days post-stress. However, given the sex difference in the magnitude of retraction, males showed a more pronounced recovery. This pattern of results suggests that following chronic stress, neurons in prelimbic cortex of both males and females demonstrate remarkable plasticity, with full recovery by 10 days post-stress.

Disclosures: K.M. Moench: None. **C.L. Wellman:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.08/CC25

Topic: E.05. Stress and the Brain

Support: ERC-2013-AdG 341116-PressBirth (KK)

Title: Arginine vasopressin at nanomolar concentrations blocks bumetanide-sensitive GDPs in the perinatal rodent hippocampus

Authors: *P. SEJA, J. LINDFORS, A. SPOLJARIC, E. RUUSUVUORI, J. VOIPIO, K. KAILA;
Univ. of Helsinki, Helsinki, Finland

Abstract: During the perinatal period, a number of allostatic physiological mechanisms are activated in mammals to facilitate the transition to extrauterine life (Tyzio et al., Science 2006). Recent studies on humans (Wellmann et al., J Clin Endocrinol Metab 2010) have demonstrated a massive release of arginine vasopressin (AVP) in the neonate during birth, but the neurobiological consequences of this mechanism have not been studied. Here, we made electrophysiological experiments on rat hippocampal slices and on whole hippocampi *in vitro* at the end of the fetal period (E20-21); within 2h after birth; and during the first two postnatal days

(P0 and P1). At all these time points, CA3-driven network events known as Giant Depolarizing Potentials (GDPs) were observed, and they were blocked by bumetanide, a selective inhibitor of Cl^- uptake by NKCC1, indicating a key role for depolarizing GABA in their generation . The perinatal depolarizing action of GABA was further confirmed by fluorescent imaging (Fluo-4AM) of intraneuronal cell-autonomous Ca^{2+} transients evoked by $GABA_A$ agonists. GDPs were strongly suppressed by exposure to 5-10 nM AVP. The AVP effect was prevented by the V1a receptor antagonist SR49059 (20 nM). The suppression of GDPs by AVP was transient with a maximum at 200-300s, and the GDPs retained their sensitivity to bumetanide after recovery in the presence of AVP. AVP induced a massive increase in the frequency of TTX-sensitive spontaneous IPSCs (sIPSCs) in CA3 pyramidal neurons both in the presence and absence of ionotropic glutamate receptor blockers. Under the latter conditions, a pronounced desynchronization of synaptic events took place, which readily explains the suppression of GDPs (Sipilä et al., J. Neurosci. 2005). Thus, we conclude that during birth, AVP exerts neuroprotective actions and prevents pathophysiological plasticity by desynchronizing neuronal network activity. This is an intriguing finding, especially in the light of studies, which show that AVP release during human birth is further enhanced under pathophysiological conditions such as birth asphyxia (Schlapbach et al., BMC Pediatrics 2011).

Disclosures: P. Seja: None. **J. Lindfors:** None. **A. Spoljaric:** None. **E. Ruusuvuori:** None. **J. Voipio:** None. **K. Kaila:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.09/CC26

Topic: F.02. Animal Cognition and Behavior

Support: HHMI Gilliam Fellowship

NIMH K01MH099371

Title: Deconstructing ventral hippocampal control of innate and learned fear behavior

Authors: *J. C. JIMENEZ[1], A. GOLDBERG[1], G. ORDEK[1], L. DREW[2], V. LUNA[1], M. WRIGHT[3], R. HEN[1], M. A. KHEIRBEK[1];
[1]Columbia Univ., New York, CA; [2]Wolfson Inst. for Biomed. Res., London, United Kingdom; [3]Stanford, Palo Alto, CA

Abstract: Recent studies implicate functional heterogeneity in projection specific populations within affective circuits, including the ventral hippocampus and amygdala. How these specialized circuits contribute differentially to anxiety and fear related behaviors, however, is not understood. In this study, we have focused on the ventral hippocampus (vHPC), and leveraged

optical technologies to determine how the extended vHPC circuit can modulate innate and learned fear behavior. In order to understand the mechanisms by which the vHPC may control anxiety-related and learned fear behavior, we have used *in vivo* functional calcium imaging and optogenetic terminal modulation in mice exploring safe and anxiogenic environments. Our imaging studies revealed that vHPC neurons exhibit a higher rate of calcium transients during exploration of innately anxiogenic environments, including the center in the Open field Test and open arms of the Elevated Plus Maze, but not during exploration of a novel object. Next, we dissected the relative contribution of vHPC outputs to innate and learned fear. We focused on outputs to two limbic regions implicated in emotional behavior, the amygdala and hypothalamus. Channelrhodopsin-assisted circuit mapping confirmed that vHPC sends a direct monosynaptic projection to the lateral hypothalamic (LH) area and the basomedial nucleus of the amygdala (BMA). Acute optogenetic modulation of vHPC-BMA terminals impaired contextual fear conditioning without impacting behavior in tests of innate anxiety. In contrast, optical activation of vHPC-LH terminals was anxiogenic and aversive, without affecting learned fear measures. These studies implicate a projection-specific functional dissociation of vHPC outputs during innate and learned fear behavior. Further, our *in vivo* imaging data indicates a specialized representation of anxiogenic environments within the local vHPC circuit. Our ongoing studies are aimed at comparing vHPC population activity during innately anxiogenic tasks to activity in contextual fear conditioning paradigms. Further, we are examining the interplay between vHPC activity and anxiety state through pharmacological and behavioral manipulations.

Disclosures: **J.C. Jimenez:** None. **A. Goldberg:** None. **G. Ordek:** None. **L. Drew:** None. **V. Luna:** None. **M. Wright:** None. **R. Hen:** None. **M.A. Kheirbek:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.10/CC27

Topic: E.05. Stress and the Brain

Support: NIH Grant R21 MH091445

NIH Grant R21 MH105846

NIH Grant R01NS066019

NIH Grant NEI EY13079

NIH Grant R01NS047557

NIH NCATS UL1 TR000038

Fullbright Graduate Study Grant

Title: alphabetadelta-GABA-A receptors at excitatory axo-spinous synapses of the dorsal hippocampal CA1 of adolescent female rats contributes towards suppression of food restriction-evoked excessive wheel running, an animal model of anorexia nervosa, but is not affected by food restriction alone or rearing in isolation and is up-regulated by exercise

Authors: *C. J. AOKI[1], T. G. CHOWDHURY[2], W. PIPER[2], Y.-W. CHEN[2];
[1]Ctr. Neural Sci., [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: We have previously shown that GABA-A receptors containing alpha4 and delta subunits (α4βδ-GABAARs) occur on spine membranes of the hippocampal CA1 of adolescent female rats forming excitatory synapses. The levels of these receptors are up-regulated in stratum radiatum of the dorsal hippocampal CA1 field within 4 days of exposure to food restriction (P41-P44), when this is combined with access to a running wheel (P36-P44). Because food restriction-induced hyperactivity captures two hallmarks of anorexia nervosa (AN) - excessive exercise and voluntary food restriction (animals choose to run, instead of eating, even during the limited periods of food access), leading to exaggerated weight loss, it has been used as an animal model for AN, called activity-based anorexia (ABA). α4 level was shown previously to be correlated negatively with the extent of hyperactivity. We have also shown that the extent of running following ABA is correlated with anxiety. We hypothesize that up-regulation of these GABAARs contribute towards anxiolysis, suppression of wheel running and protection from excessive weight loss. In order to evaluate the specificity of the ABA induction, we determined whether wheel running alone (EX) or food restriction alone (FR) also up-regulates α4βδ-GABAARs. Moreover, since animals had to be housed in isolation in order to measure wheel activity, we also tested whether isolated housing contributes towards the up-regulation. These questions were analyzed by repeating the electron microscopic immunocytochemical analysis to quantify α4 subunit immunoreactivity (ir) at 200 randomly sampled spines from the same brain region of 7 FR, 7 EX, 8 CON (neither food restricted nor given access to a running wheel), 8 isolated-CON and 8 pair-housed-CON age-matched animals. We observed no effect of isolated housing. The averaged values of α4-ir across the animals were significantly elevated for the EX group (67%), even more than for the ABA group (37%), relative to CONs, while FR did not evoke an elevation (-9%, relative to CONs). Running on the first day of food restriction correlated strongly and negatively with α4 level among the ABA animals (R=-0.84, p=0.009), recapturing what we observed in a previous cohort, but this correlation was absent for the equivalent day's running of the EX group (R=0.25, p=.59). Instead, EX group's α4 level correlated strongly and positively with their running on days 2 plus 3 of wheel access (R=0.84, p=0.02). These findings confirm that α4βδ-GABAARs in the dorsal hippocampus contribute towards suppression of food restriction-evoked excessive exercise but also supports motor learning on the wheel, when food restriction stress is absent.

Disclosures: C.J. Aoki: None. **T.G. Chowdhury:** None. **W. Piper:** None. **Y. Chen:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.11/CC28

Topic: E.05. Stress and the Brain

Support: NIH Grant R21 MH091445

NIH Grant R21 MH105846

NIH Grant R01NS066019

NIH Grant NEI EY13079

NIH Grant R01NS047557

NIH NCATS UL1 TR000038

Fullbright Graduate Study Grant

Title: Immunoreactivity for the NR2A and NR2B subunits of NMDA receptors is increased at axo-spinous synapses of hippocampal CA1 of adolescent female rats exhibiting food restriction-evoked hyperactivity, an animal model of anorexia nervosa

Authors: *L. KLINGENSMITH, H. ACTOR-ENGEL, Y.-W. CHEN, T. G. CHOWDHURY, C. AOKI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Adolescence is a developmental period of increased vulnerability to psychological disorders and specifically eating disorders. Anorexia Nervosa (AN) is a neuropsychiatric disorder of unknown etiology, more common among females than males, with the first onset usually around puberty. AN is characterized by self-induced restricted caloric intake and an extreme pursuit of thinness that ultimately leads to emaciation, and in some cases death. Due to recorded changes in cognitive function and synaptic plasticity during puberty, we sought to investigate whether the expression of NR2B and NR2A subunits of the N-methyl-D-aspartate receptor (NMDAR) changed in the CA1 region of the adolescent females' dorsal hippocampus in response to the induction of an animal model of AN, Activity-Based Anorexia (ABA). ABA induction involves exposing animals to running wheel access for 8 days (P36-P44) and food restriction (FR) during the last 4 of those days (P41-P44). ABA captures two hallmarks of AN - over-exercise and voluntary FR. We used electron microscopic (EM) immunocytochemistry to quantify changes in the level of NR2B and NR2A immunoreactivity (ir) at synaptic and non-synaptic sites of axo-spinous junctions. We analyzed at least 100 synapses from each of 8 ABA and 4 Control animals. NR2A- and NR2B-subunit ir was significantly greater post-synaptically for synapses of ABA animals compared to Controls', using post-embed gold immunolabel (PEG) as the unit for counting to quantify levels of ir or synapse as a unit, to quantify the proportion of ir synapses. Both the NR2A- and NR2B-ir were driven by those animals that responded to FR with higher activity (ABA-High). In addition, NR2B-NMDARs were also increased pre-

synaptically for the ABA tissue, driven by the ABA-Low animals that responded minimally to FR. When non-synaptic zones were included, both subunits were higher within axon terminals of ABA-Low animals. These findings suggest that the experience of FR-evoked hyperactivity of ABA increases NMDAR-mediated synaptic plasticity. However, the increased excitation at spines would increase excitability of the hippocampus, and this may be contributing to stress-induced anxiety as well as increased vulnerability to ABA. Finally, presynaptic NR2B expression correlated negatively with running activity two days prior to food restriction and on the first day of FR, suggesting that these receptors participate in animals' resilience to the maladaptive behavior of FR-evoked hyperactivity.

Disclosures: L. Klingensmith: None. **H. Actor-Engel:** None. **Y. Chen:** None. **T.G. Chowdhury:** None. **C. Aoki:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.12/CC29

Topic: F.02. Animal Cognition and Behavior

Support: St. Mary's College of Maryland

Title: Habituation and fear conditioning in neonatal ventral hippocampal lesioned rats

Authors: *W. P. JORDAN[1], C. DI LEO[2];
[1]St Mary's Col. of MD, St Marys City, MD; [2]Psychology, St Mary's Col. of MD, St. Mary's City, MD

Abstract: The neurodevelopmental aspects of schizophrenia are modeled in rats with lesions to the hippocampus early in life (Lipska & Weinberger, 2000). A variety of behavioral abnormalities emerge post-adolescence (see Tseng, Chambers, & Lipska, 2009 for a review). Animals with NVHL lesions have been found to show deficits in Pavlovian fear conditioning (e.g., Gilmartin, Balderston, & Helmstetter, 2014) and its extinction (Quirk, Garcia, & Gonzalez-Lima, 2006)--deficits also found in human schizophrenic patients (Braff, Saccuzzo, & Geyer, 1991). The present study looked at lick suppression habituation to a noise CS in NVHL-lesioned animals, followed by fear conditioning and then extinction. 7 Sprague-Dawley rats received bilateral ibotinic acid lesions to the hippocampus on post-natal day 7, while 8 rats received sham lesions or were unoperated controls. In adulthood rats were water deprived and trained to lick. Habituation training consisted of 3 daily presentations of a noise stimulus (88dB, 30s) while the animal was licking. Rats then were conditioned to fear the noise CS by paired it with foot shock (0.5mA, 0.5s). One CS/US pair was given each day until each rat's licking during the CS met a suppression criterion. Conditioning was followed by 8 extinction sessions. Both groups showed

equal suppression to the first CS presentation, but Control animals showed more long-term habituation across days. Across the five conditioning sessions, Control animals showed robust fear conditioning. NVHL animals also showed significant conditioning and reached a common asymptote to the Controls, but the unequal starting points produced a significant group by day interaction. During extinction, the two groups initially were equally suppressed, but Control animals were faster to extinguish producing significant group differences before reaching a common extinction asymptote. The habituation deficit in NVHL animals makes difficult the interpretation of the differences during conditioning. The habituation deficits in NVHL-lesioned animals are of interest theoretically and empirically, as the animal hippocampal literature often makes a distinction between habituation within different response systems such as exploration, startle, and lick suppression (e.g., Leaton, 1981). These potential deficits in habituation, fear conditioning, and extinction are compatible with reports from human schizophrenics, although it is difficult to attribute the deficits to any one of these three learning processes or to a more general problem with behavioral perseveration.

Disclosures: **W.P. Jordan:** None. **C. Di Leo:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.13/CC30

Topic: E.05. Stress and the Brain

Support: CONACyT Grant 221092

Title: Astroglial changes in the hippocampus of aged rats exposed to chronic variable stress

Authors: ***D. L. MORAN TORRES**[1], D. RUESGA-BARCENAS[1], S. LUQUIN[1], J. GARCÍA-ESTRADA[2], R. RAMOS-ZUÑIGA[1], F. JAUREGUI-HUERTA[1];
[1]Neurociencias, Univ. De Guadalajara, Guadalajara, Mexico; [2]Neurociencias, Ctr. de Investigación Biomédica de Occidente. IMSS, Guadalajara, Mexico

Abstract: In this study, we exposed adult aged rats to a chronic variable stress model and compare astroglial changes as a function of wether or not the rats were exposed to stress early in their lives. Experiments were carried out on 18-month-old male rats divided in 4 groups as follows: Control (old rats under standard laboratory conditions), Early-life stress (old rats who were exposed to environmental noise from postnatal days 21 to 35), Chronic variable stress + Early-life stress (old rats exposed to a chronic stress protocol which previously were exposed to the early-life noise stress) and Chronic variable stress (old rats who were exposed only to the chronic stress protocol). Immunohystochemistry against BrdU+GFAP was conducted in the hippocampal regions DG, CA3, CA2 and CA1. We confocally analyzed astrogenic

(BrdU+GFAP labeled cells) and morphological (GFAP labeled cells) changes produced by these conditions. We found with this procedure that senile rats exposed to chronic stress, improved their astroglial proliferative rate when this experience was preceded by early life stress. Region specific changes were also observed on astroglial morphology. Then, our results support the idea that previous exposure to stressing agents may exert important modulating effects on astroglial activity of aged subjects.

Disclosures: D.L. Moran Torres: None. **D. Ruesga-Barcenas:** None. **S. Luquin:** None. **J. García-Estrada:** None. **R. Ramos-Zuñiga:** None. **F. Jauregui-Huerta:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.14/CC31

Topic: E.05. Stress and the Brain

Title: The effect of the swearing on hippocampal subregions in adolescence

Authors: *D. KIM, J. YOO, S. LEE, B. JEONG;
KAIST, Daejon-City, Korea, Republic of

Abstract: Introduction The peer verbal abuse (PeVA) is serious and widespread problem in adolescence. It can damage not only emotion but also brain, especially hippocampal regions. However most adolescent frequently use swear words without recognition of the detrimental effects of swearing. In our study, various analytic methods were applied to find the negative impacts of PeVA on the structural changes in the hippocampus and its subregions, and we also tried to determine relationships between the structural changes of hippocampus and behavioral measurements including depression and anxiety to verify the meaning of structural changes **Method** We enrolled 43 high school students. They were classified into 2 groups according to score of Verbal abuse Questionnaire (VAQ), the cut-off value of which was 40. We obtained clinical and MRI data. Some subjects, who has depression or organic brain problem were excluded from study. Finally 31 subjects was enrolled study (PeVA group 15, control group 16). The Hippocampal subfield tool from Freesurfer software was used for segregating each hippocampal subregion including fimbria, CA2_3, CA1, CA_DG, Presubiculum, subiculum, hippocampus. Data from each participant were entered into general linear model, using R software 3.0.1 to obtain volume of each subregion. Effects of IQ score of subject and internal cranial volume were corrected, as they can affect volume of hippocampus. Correlation analysis was conducted to identify correlation of hippocampal volume with severity of parental verbal abuse (PVA) and PeVA, using R software 3.0.1 **Results** We found that the volume of left hippocampus and subiculum was negatively related to severity of PeVA(Fig.1). Volume of left hippocampus and subiculum in PeVA group was smaller than that in normal control. The

hippocampus volume changes were associated with individual's anxiety trait in adolescence who reported substantial PeVA experiences. PVA did not have significant correlation with volume of hippocampal region **Discussion** Correlation between hippocampal volume and severity of PeVA show that quantity of PeVA play a role in hippocampal damage. Volume reduction only in the left sided hippocampus might suggest that verbal memory was processed at left hippocampus. Volume reduction in subiculum may relate to change of level of stress hormone, because subiculum mediate hypothalamic-pituitary-adrenal axis. We identified structural effect of swearing on hippocampus in this study. We also need to identify functional effect of swearing in the future.

Disclosures: D. Kim: None. **J. Yoo:** None. **S. Lee:** None. **B. Jeong:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.15/CC32

Topic: E.05. Stress and the Brain

Support: NS28912

MH73136

NS045260

Title: A role for endogenous CRH in the hippocampus?

Authors: *B. G. GUNN, Y. CHEN, G. LYNCH, T. Z. BARAM;
Univ. of California Irvine, Irvine, CA

Abstract: Rationale: The release of corticotropin-releasing hormone (CRH) from neuroendocrine cells of the hypothalamic paraventricular nucleus is integral to the initiation of the stress response. However, CRH is additionally expressed in a number of brain regions including the hippocampus, amygdala and bed nucleus of the stria terminalis. Hippocampal functions are impacted by stress, suggesting a potential role of hippocampal CRH (Joels & Baram, 2009). CRH is expressed in hippocampal interneurons in CA1 and CA3, whilst the receptors for this peptide (CRHR1 and CRHR2) appear to be located almost exclusively in discreet subcelluar domains on pyramidal cells. In addition, exposure to stress results in the release of hippocampal CRH (Chen et al, 2012) and contribution by the peptide to the stress-related effects on memory (Chen et al, 2010). In slice preparations, exogenous CRH results in an increase in excitability of hippocampal pyramidal cells (Aldenhoff et al, 1983; Hollrigel et al, 1998). Here we explore the notion that endogenous CRH may play a role in modulating neurotransmission within the hippocampus. Methods: C57BL/6 mice (P21-P40) were

decapitated, the brains dissected and horizontal brain slices (300-350 μm) prepared using standard procedures. The whole-cell voltage-clamp configuration was applied to record spontaneous excitatory postsynaptic currents (sEPSCs) from CA3 pyramidal cells. The presence of an endogenous CRH tone was determined by the bath application of the competitive, non peptide antagonist, NBI 30775 (1 μM). Results: In our first set of experiments, blocking the actions of endogenous CRH reduced the frequency of sEPSCs recorded from CA3 pyramidal cells, whilst having only modest effects upon the peak amplitude and decay kinetics of these currents. Additional experiments are ongoing. Conclusions: This observation would suggest that endogenous CRH, released from a population of hippocampal interneurons, modulates excitatory synaptic transmission in an acute brain slice preparation. This work was supported by NS28912, MH73136, and NS045260. References: Aldenhoff, J.B., Gruol, D.L., Rivier, J., Vale, W., and Siggins, G.R. (1983). Science 221, 875-877. Chen, Y., Andres, A.L., Frotscher, M., and Baram, T.Z. (2012). Front Cell Neurosci. 6: 13. Chen, Y., Rex, C. S., Rice, C. J., Dubé, C. M., Gall, C. M., Lynch, G., and Baram, T. Z. (2010). Proc.Natl.Acad.Sci.U.S.A. 107, 13123-13128. Hollrigel, G.S., Chen,K., Baram, T.Z., and Soltesz, I. (1998). Neuroscience 84, 71-79. Joëls, M., and Baram, T.Z. (2009). Nat.Rev. Neurosci. 6, 459-466.

Disclosures: **B.G. Gunn:** None. **Y. Chen:** None. **G. Lynch:** None. **T.Z. Baram:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.16/CC33

Topic: E.05. Stress and the Brain

Support: DARPA W911NF1010093

Title: Interleukin-1 α in the ventral hippocampus mediates aspects of stress vulnerability

Authors: *J. PEARSON-LEARY[1], D. EACRET[1], R. CHEN[1], L. WILSON[1], S. BHATNAGAR[1,2];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Chronic psychological stress is associated with cognitive, emotional, and physical disturbances. Several immune changes have been observed in the brains of animals subjected to chronic stress. Importantly, some animals are resilient to the effects of chronic stress, while other animals are vulnerable. Using an adult male rat model of social defeat stress we have identified subpopulations of rats that are either vulnerable (short-latency; SL) or resilient (long-latency; LL) to the effects of social defeat stress. Behaviorally, vulnerable SL rats have increased depressive- and anxiety-like behaviors, while resilient LL rats are no different from controls. PCR array analyses identified changes in patterns of immune signaling molecules in the ventral

hippocampus (vHPC) of SL and LL rats. Of specific interest, interleukin-1 alpha (IL-1α) and its receptor interleukin-1 receptor type 1 (IL1R1) were increased in the vHPC of SL rats relative to LL rats. To determine whether vHPC IL-1α was important for stress vulnerability, we delivered recombinant IL-1α or recombinant interleukin-1 receptor antagonist (IL1-RA; an endogenous antagonist to interleukin-1) to the vHPC of rats 1 h prior to social defeat stress for 5 d. As expected, IL-1α lowered defeat latencies consistent with the vulnerable phenotype while IL1-RA increased defeat latencies consistent with the resilient phenotype. Rats treated with intra-vHPC IL-1α were not different from controls during a social interaction task of anxiety-like behaviors, while IL-1RA-treated rats showed decreased anxiety-like behaviors relative to control and IL-1α-treated rats. Analyses of brains from treated rats showed increased blood vessel density and microglial activation in vHPC of IL-1α-treated rats relative to control and IL1-RA-treated rats. These data suggest that while IL-1α treatment was sufficient to activate inflammation in the vHPC and increase some behaviors associated with stress vulnerability, IL-1α was insufficient in producing the anxiety-like phenotype that is known to occur with stress vulnerability. Taken together, our data suggest that interleukin-1 signaling in the vHPC is involved in some components of stress vulnerability.

Disclosures: J. Pearson-Leary: None. **D. Eacret:** None. **R. Chen:** None. **L. Wilson:** None. **S. Bhatnagar:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.17/CC34

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: The hippocampus is not required for context discrimination in a pavlovian fear conditioning task

Authors: *J. Q. LEE, R. J. SUTHERLAND, R. J. MCDONALD;
Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: The hippocampus is thought to be important for discriminating between contexts, but not for basic context conditioning. Results are mixed on whether increased amounts of training can overcome context discrimination deficits in rats with hippocampal damage. Notably, studies that support the idea that increased training can overcome deficits did not examine lesion extent. Thus, spared hippocampal tissue might have supported context discrimination. In order to resolve these inconsistencies, rats with complete hippocampal lesions (> 75%) were trained for one or three conditioning sessions in a discriminative fear conditioning task. Rats with

hippocampal damage demonstrated clear discriminative freezing between shock-paired and unpaired contexts and a weaker preference for the unpaired context. In subsequent experiments, the parametric limitations of hippocampal and extra-hippocampal contributions to context discrimination in both anterograde and retrograde directions, and the discriminanda that influence these abilities were also assessed. Our findings contribute to recent advancements in understanding the role of the hippocampus in long-term memory for emotional events and the specific places in which they occur.

Disclosures: J.Q. Lee: None. **R.J. Sutherland:** None. **R.J. McDonald:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.18/CC35

Topic: E.05. Stress and the Brain

Support: PD/BD/105915/2014

PTDC/SAU-NMC/118971/2010

NIH R01 NS056049

NIH P50 AG008702

Title: Characterization of the lipidome along the rat hippocampal longitudinal axis

Authors: *A. M. MIRANDA[1,2,3,4], R. B. CHAN[3,4], F. V. BRAVO[1,2], B. ZHOU[3,4], V. PINTO[1,2], G. DI PAOLO[3,4], N. SOUSA[1,2], T. G. OLIVEIRA[1,2];
[1]Life and Hlth. Sci. Res. Inst., Braga, Portugal; [2]Sch. of Heath Sciences, Univ. of Minho, Braga, Portugal; [3]Dep. of Pathology and Cell Biol., [4]Taub Inst. for Res. on Alzheimer's Dis. and the Aging Brain, Columbia Univ. Med. Ctr., Nyc, NY

Abstract: The hippocampus is a complex brain structure involved in learning, memory and emotional responses and its functioning is affected in various brain disorders. Despite conserved intrinsic circuitry, behavioral and anatomical studies suggest the existence of discrete regions along its longitudinal axis, each orchestrating distinct cognitive operations and accounting for its functional diversity. More recently, high-resolution gene expression analysis identified multiple, spatially segregated molecular domains and subdomains. Lipids are major constituents of the brain and growing evidence implicates their role in physio- and pathological processes. Little is known about the brain's lipidome, particularly of the hippocampus along its longitudinal axis. Here, we used an unbiased mass spectrometry approach to characterize the lipid composition of the dorsal (DH) and ventral (VH) areas of the rat hippocampus. Additionally, we evaluated each region's functional flexibility to modulate its composition under treatment with corticosterone, a

model for chronic exposure to stress. We confirmed a conserved intrinsic composition of both DH and VH relative to other brain areas. However, both domains differed significantly in sphingolipid and phospholipid metabolism, suggesting differential regulation of lipid signaling pathways. Additionally, we found that while overall biochemical coherence between both areas of the hippocampus is conserved under stressful conditions, they present identical responses to some extent. Most strikingly, cholesterol esters and ceramide accumulate upon exposure to high corticosterone levels. Diacylglycerol and phosphatidic acid present region-specific fatty-acyl profiles suggesting a differential impact on their metabolizing enzymes along the hippocampal axis. Moreover, lipid changes linearly correlate with blood corticosterone levels ($R2>0.4$). Our results confirm a multipartite view of the hippocampus based on its lipid signature. These findings highlight the importance of understanding lipid metabolism in the context of brain function with potential implications to disorders such as chronic exposure to stress and Alzheimer's disease. A thorough knowledge of the boundaries within the hippocampal axis will provide new tools to understand and manipulate each region's function, both in health and disease.

Disclosures: **A.M. Miranda:** None. **R.B. Chan:** None. **F.V. Bravo:** None. **B. Zhou:** None. **V. Pinto:** None. **G. Di Paolo:** None. **N. Sousa:** None. **T.G. Oliveira:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.19/CC36

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1R15MH100689-01

Title: Unpaired fear conditioning yields hippocampus-dependent responding

Authors: M. R. HERBST[1], A. F. POSTLE[1], M. S. FANSELOW[2], *J. J. QUINN[1]; [1]Psychology, Miami Univ., Oxford, OH; [2]Psychology, UCLA, Los Angeles, CA

Abstract: The hippocampus (HPC) plays a critical role in the formation of conjunctive representations among stimuli. This may explain why manipulations of HPC function alter learning in a variety of procedures including the formation of spatial maps, unified representations of context, and episodic memories. Using a backward trace fear conditioning procedure, we previously showed that responding to a tone that was not explicitly paired with footshock produced a moderate level of freezing when tested later. In addition, this freezing was eliminated by post-training neurotoxic lesion of the dorsal HPC (DH). The present experiments were designed to assess whether other explicitly unpaired conditioning procedures similarly yield HPC-dependent responding. Five conditioning procedures were used: footshock US-only,

intermixed, backward trace, tones→footshocks, footshocks→tones. Thus, all rats received ten tones (except US-only) and ten footshocks, but the order of stimulus presentation within the session differed between groups. One day following training, half of the rats in each training condition were given a neurotoxic, NMDA-induced lesion of the DH while the others received a sham surgery. Seven to ten days following surgery, rats were tested for freezing to both the original training context and to the tone in a novel context. Results from Experiment 1 indicate a high level of HPC-dependent context freezing in all groups. Further, there was significant freezing to the tone in all groups that had received the tone during conditioning, but not in the US-only group. Tone freezing was also HPC-dependent. One concern was the high level of baseline freezing that was observed in the novel, tone test context. Therefore, Experiment 2 was identical to Experiment 1 except that three exposures to the tone test context were introduced prior to the tone test. These context exposures significantly reduced baseline freezing (to near-zero levels) in all groups. Despite this very low baseline freezing, tone and post-tone freezing was observed in all groups and this responding was HPC-dependent. The data will be discussed in terms of the formation of a HPC-dependent conjunctive representation of the conditioning episode.

Disclosures: **M.R. Herbst:** None. **A.F. Postle:** None. **M.S. Fanselow:** None. **J.J. Quinn:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.20/CC37

Topic: F.02. Animal Cognition and Behavior

Support: SDSU Oscar Kaplan Postdoctoral Fellowship in Developmental Issues

Title: Digital fear conditioning in rats: utilizing LCD-based visual context manipulations during conditioning

Authors: ***N. J. MURAWSKI**[1], A. ASOK[2];
[1]Ctr. For Behavioral Teratology, San Diego, CA; [2]Dept. of Psychological and Brain Sci., Univ. of Delaware, Newark, CA

Abstract: Contextual fear conditioning (CFC) is a powerful behavioral assay for examining the neurobiology of learning and memory. The simple behavioral setup and robust long-lasting memories formed during conditioning have made CFC an ideal paradigm for incorporating methodological innovations in behavioral neuroscience. CFC is highly context-specific in that higher levels of conditioned freezing are observed when training and testing occur in the same, versus a different, context. Discriminating between similar, but not identical contexts requires

neural processes that are distinct from those required for discriminating between two non-similar contexts (Nakashiba et al., 2012, Cell 149: 188-201). However, experimental differences between "similar" and "non-similar" contexts can be ambiguous: Typically, one context is made distinct from another by manipulating one or more features of a context (e.g., spatial, visual, tactile, or olfactory) - similar contexts share more features than non-similar contexts. Developing new methodologies to systematically alter contexts can improve experimental control over context features and help better identify the neural substrates that support contextual fear conditioning. Previous research demonstrates that rats can utilize changes in visual contexts projected onto LCD screens to perform a hippocampus-dependent response selection task (Kim, et al. 2012, Frontiers in Behavioral Neuroscience 6: 1-10). Here we ask if adult male rats can show the context pre-exposure facilitation effect by utilizing visual contextual information provided by four LCD screens surrounding a conditioning chamber. Rats received a 5-min pre-exposure session on Day 1, an immediate 1.5 mA foot shock on Day 2, and a 5-min context test on Day 3. Rats were either pre-exposed to Context A or B on Day 1. Context A and Context B only differed in the visual image projected onto the LCD monitors. Immediate shock and context test occurred in Context A. Rats pre-exposed to Context A froze significantly more than rats pre-exposed to Context B during testing. These results indicate that rats 1) can learn fear to a visual context, 2) that changes to a visual context mediate differential conditioning, and 3) that visual features of the context can be parametrically controlled via LCD screens. Quantitative control and temporal precision of contextual features afforded by digital context presentations can greatly aid researchers in their understanding of discriminating and generalizing fear to aversive contexts.

Disclosures: N.J. Murawski: None. **A. Asok:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.21/CC38

Topic: E.05. Stress and the Brain

Support: NSF Graduate Research Fellowship

Title: Differential myelination in individual responses to stress and stress-induced anxiety

Authors: *K. LONG[1], D. KAUFER[2];
[1]UC Berkeley, Berkeley, CA; [2]Integrative Biol., Univ. of California, Berkeley, Berkeley, CA

Abstract: Individuals show a spectrum of responses to traumatic stress, ranging from normal behavior to extreme anxiety and post-traumatic stress disorder. Animal models have begun to elucidate the neural underpinnings of such disparate responses; for example, extremely affected

individuals show dendritic atrophy of hippocampal pyramidal cells, while minimally affected individuals show arborization that is indistinguishable from unexposed controls[1]. Literature from human patients suggests that, in addition to changes to gray matter, trauma and subsequent PTSD may also be associated with increases in hippocampal white matter. In addition, our lab has shown that stress causes an increase in oligodendrogenesis and myelination in the rat hippocampus[2]; however, whether effects on myelination and white matter are correlated with the spectrum of responses to stress is not understood. In this study, we used a rodent model of PTSD to evaluate myelination in extremely affected and minimally affected individuals, with the hypothesis that extremely affected individuals will show increased hippocampal myelination. Adult, male Sprague-Dawley rats were exposed to three hours of immobilization and fox urine. Seven days post-stress, animals were evaluated for anxiety behavior on the open field, elevated plus maze, and acoustic startle response and subsequently classified into the behavioral profiles of minimal behavior response (MBR) and extreme behavioral response (EBR) based on cutoff behavioral criteria[1]. Fourteen days after stress, animals were sacrificed and brains perfused for immunohistochemical analysis of oligodendrocytes and myelination. The results presented will demonstrate the role of myelination in individual stress reactivity and open the door to studies of the causal role of myelination in stress-induced anxiety. 1. Cohen *et al*., 2014. *Eur Neuropsychopharmacol*, 24(1925,1944). 2. Chetty *et al*., 2014. *Mol. Psychiatry*, (1-9).

Disclosures: K. Long: None. **D. Kaufer:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.22/CC39

Topic: F.02. Animal Cognition and Behavior

Support: McKnight Foundation

Whitehall Foundation

Title: Contextual representations along the proximodistal axis of CA1

Authors: Y. NAKAZAWA, K. TANAKA, A. PEVZNER, *B. J. WILTGEN;
Ctr. for Neurosci., UC Davis, Davis, CA

Abstract: Neurons in the rodent hippocampus form place fields that are stable in a given environment. Activation of these cells is assumed to mediate the encoding and retrieval of spatial/contextual memories. However, space is not uniformly represented across the proximodistal axis of the hippocampus, giving rise to potential functional differences. For example, place cells in proximal CA1 (pCA1) are more spatially selective than those in distal CA1 (dCA1). This difference could be explained by the unique connectivity that each of these

regions has with the entorhinal cortex (EC). pCA1 is reciprocally connected with the medial portion of the EC (MEC), which exhibits spatially selective firing (e.g. grid cells). In contrast, dCA1 is connected with the lateral EC (LEC), which is only weakly modulated by spatial inputs. Based on these differences, it is likely that proximal and distal CA1 make unique contributions to learning and memory retrieval. To examine this idea, we used TetTag mice to determine the stability of context representations in each of these regions. In our first experiment, active neurons were tagged during context fear conditioning with the long-lasting protein H2B-GFP. During subsequent testing, we found that tagged neurons in pCA1 were significantly more likely to be reactivated (measured via c-Fos expression) than cells in dCA1. This same effect was observed 1) when mice were exposed to the same context twice in the absence of shock and 2) when mice received multiple fear conditioning tests in the same environment. Subsequent analyses also revealed greater than chance reactivation in regions connected to pCA1 (MEC and distal CA3) but not those connected to dCA1 (LEC and proximal CA3). We are in the process of conducting fiber sparing NMDA lesions of proximal and distal CA1 to determine their functional contributions to the retrieval of context fear memories.

Disclosures: **Y. Nakazawa:** None. **K. Tanaka:** None. **A. Pevzner:** None. **B.J. Wiltgen:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.23/CC40

Topic: F.02. Animal Cognition and Behavior

Support: McKnight Endowment for Neuroscience

Whitehall Foundation

National Science Foundation Graduate Research Fellowship

Title: The influence of environmental parameters on hippocampal reactivation

Authors: *A. HAMIDI[1], B. J. WILTGEN[2], A. PEVZNER[3];
[2]Psychology, [3]Neurosci., [1]Univ. of California At Davis, Davis, CA

Abstract: The hippocampus plays a key role in the generation and retrieval of contextual representations. Previous research has shown that hippocampal place cells are sensitive to changes in physical parameters of the environmental context (Burgess et al, 2006). To visualize the recruitment of hippocampal cells during context exposure, our lab uses the H2B-GFP-TetTag mouse, which relies on the temporally specific activation of IEGs. Successful memory retrieval is cellularly indexed by tagging active cells, during two different context exposure sessions, with H2B-GFP and c-fos, respectively. Cells that co-localize H2B-GFP and c-

fos are operationally defined as “reactivated”. Given that neural reactivation is a commonly used cellular metric of memory retrieval, understanding how this measure varies based on the features of the testing environment is key for the consideration of future project designs. Previous data from our lab has shown that reactivation of cells in CA1 is correlated with expression of a hippocampal dependent context memory (Tanaka et al, 2014). The parameters of the environmental context, which drive the observed reactivation, however, have not yet been fully explored. Recently, we demonstrated that two exposures to large environment did not reliably result in above chance reactivation in proximal CA1 (pCA1). Neither an increase in time spent on the arena, nor spatial training on the large environment, was sufficient to drive increased reactivation. Surprisingly, the addition of boundaries in the large environment was able to rescue the low reactivation levels in pCA1. (Pevzner et al, 2014). Notably, these reactivation levels were still lower than those generated from our group’s previous data exposures to a fear-conditioning chamber (a substantially smaller environment), suggesting that area is also an important factor (Nakazawa et al, 2013). In this experiment, we have extended previous findings by investigating the effects of adding borders to a small environment (the same surface area as the fear conditioning chamber) and compared this to a small environment without borders. Future experiments will be aimed at investigating whether the addition or removal of environmental boundaries within the large and small environmental arenas can further drive levels of cellular reactivation in the hippocampus.

Disclosures: **A. Hamidi:** None. **B.J. Wiltgen:** None. **A. Pevzner:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.24/CC41

Topic: F.02. Animal Cognition and Behavior

Support: NIH MH081968

NIH MH096274

NIH F31 MH102041

National Science Foundation

Title: Direct hippocampal-prefrontal input is required for anxiety-related neural synchrony and behavior

Authors: ***N. PADILLA**[1], S. S. BOLKAN[1], G. M. PIERCE[1], D. R. BLACKMAN[3], T. SPELLMAN[1], J. A. GORDON[1,2];

[1]Neurobio. and Behavior, [2]Dept. of Psychiatry, Columbia Univ., New York, NY; [3]Barnard Col., New York, NY

Abstract: The ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) are both required for the expression of anxiety-like behavior in rodents. Theta-frequency (4-12 Hz) synchrony within this circuit correlates with anxiety-like behavior. Moreover, cells in the mPFC that encode the aversive structure of the environment are phase locked to theta oscillations in the vHPC. We hypothesized that the direct vHPC input to the mPFC is necessary for avoidance behavior, anxiety-induced theta synchrony and mPFC encoding of aversion. To address this hypothesis, multi-site neural recordings were obtained while optogenetically inhibiting the direct vHPC-to-mPFC projection. Previously we have demonstrated that inhibition of vHPC input to the mPFC during the elevated plus maze (EPM) decreased avoidance of the open arms, as well as theta synchrony between mPFC and vHPC (Padilla et al. 2014, SfN). Here we demonstrate that this effect is frequency specific, as there were no changes in synchrony in delta (1-4 Hz), beta (12-30 Hz), or gamma (30-70 Hz) frequency ranges. Moreover, inhibition of vHPC-mPFC pathway did not affect theta synchrony between BLA-mPFC and BLA-vHPC. Inhibition of the vHPC input to the mPFC also disrupted arm-type encoding by mPFC single units. Interestingly, inhibition of this pathway in a non-aversive maze also disrupted arm-type encoding, suggesting that the vHPC input to the mPFC is necessary for representation of both aversive and non-aversive contexts within the mPFC. Finally, silencing another major excitatory input to the mPFC, the medial dorsal thalamic input, did not disrupt avoidance behavior nor disrupted mPFC encoding of the EPM. These results reveal a frequency- and pathway-specific role for the vHPC-mPFC projection in anxiety-related vHPC-mPFC synchrony and the spatial encoding of aversive information within the mPFC.

Disclosures: **N. Padilla:** None. **S.S. Bolkan:** None. **G.M. Pierce:** None. **D.R. Blackman:** None. **T. Spellman:** None. **J.A. Gordon:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.25/CC42

Topic: F.02. Animal Cognition and Behavior

Support: NIH MH081968

Title: Immunohistological quantification of parvalbumin- and somatostatin-expressing interneuron activity during fear expression and extinction

Authors: S. GOLDBERG[1], J. M. STUJENSKE[2], *J. A. GORDON[3];
[1]Biol., Barnard Col., New York, NY; [2]Columbia Univ., New York, NY; [3]Columbia Univ/NYSPI, New York, NY

Abstract: Inhibitory circuits have been implicated in controlling the expression and extinction of fear memories. In particular, interfering with GABAergic signaling in the basolateral amygdala (BLA) has been shown to alter the expression and extinction of fear. However, the role of specific BLA interneuron subtypes in regulating the expression and extinction of fear memories is not well understood. In order to investigate the relevance of two major BLA interneuron subtypes, parvalbumin-positive (PV+) and somatostatin-positive (SOM+) neurons, to extinction learning, we quantified cellular activity during fear expression and extinction using c-Fos expression. C57Bl/6 mice were trained to associate a tone (CS+) with a mild foot-shock. They were divided into three groups for testing: a “fear group” that was exposed to a CS- followed by the CS+, an “extinguished group” multiply re-exposed to the CS+, and a “control” group that heard only the CS-. Tissue was harvested 2 hours after exposure and processed for immunstaining for c-Fos, PV, and SOM. Preliminary data show increased c-Fos/SOM co-labeling in the BLA from mice in the extinguished group and the control group compared to that of the fear group ($p<.05$). These findings suggest that SOM+ interneurons decrease activity during fearful states. Unexpectedly, there was no significant difference in c-Fos co-labeling with PV between the three groups. These data suggest that SOM+ interneuron activity may be downregulated during the expression of learned fear, and that this downregulation is reversed during extinction.

Disclosures: S. Goldberg: None. **J.M. Stujenske:** None. **J.A. Gordon:** None.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.26/CC43

Topic: F.02. Animal Cognition and Behavior

Support: R01-MH104344

R01-MH071916

R21-MH101579

Title: Attenuating the mania-like hyperactive, risk-preferring, and high motivation behavioral profile of mice with low dopamine transporter levels using a dopamine D1 receptor antagonist

Authors: *M. B. MILIENNE-PETIOT[1,2], M. A. GEYER[1,3], J. W. YOUNG[1,3];
[1]Psychiatry, UCSD, La Jolla, CA; [2]Div. of Pharmacol., Utrecht Univ., Utrecht, Netherlands;
[3]Res. Service, VA San Diego Healthcare Syst., San Diego, CA

Abstract: Introduction - Bipolar disorder (BD) is a serious mental illness characterized by switches between episodes of the low-energy/amotivation state of depression and the abnormally high-energy/risk-taking state of mania. These behavioral domains can be quantified using cross-species tests such as the Iowa Gambling Task (IGT), behavioral pattern monitor (BPM), and progressive ratio breakpoint (PRB). Mice with reduced dopamine transporter (DAT) levels (knockdown; KD) exhibit abnormal behavior consistent with mania patients. Some forms of reward-seeking behavior can be attenuated via dopamine D1 receptor (DRD1) antagonist treatment. Hence, we hypothesized that treatment with the (DRD1) antagonist SCH23390 (SCH) would attenuate mania-relevant behaviors of DAT KD mice while not affecting wild-type (WT) mice. Methods - Sixty male DAT KD and WT littermates were trained to nose-poke in 5-choice operant chambers and then treated with 0.03 or 0.01 mg/kg SCH or vehicle 10 min prior to testing in a within-subjects design for the IGT, and between-subjects design for the PRB and BPM. Results - DAT KD and WT good performers increased their selection of advantageous choices from the first to the last 20-min time bin [$F(2;5) = 67.9$, $p<0.05$] and [$F(2;4) = 16.4$, $p<0.01$] respectively. However, good-performing DAT KD tended to make fewer advantageous choices compared to WT [$F(1,46) = 3.8$, $p=0.08$]. SCH treatment pushed good and bad performing mice to chance levels. The highest SCH dose reduced impulsivity in good-performing DAT KD mice as measured by the % premature responses [$F(2;5) = 6.6$, $p<0.05$]. The %omission and mean choice latency were higher at this dose in the DAT KD compared to vehicle [$F(2;5) = 16.2$, $p<0.05$] and [$F(2;5) = 8.7$, $p<0.01$] respectively. Additionally, SCH reduced activity [$F(2;48) = 35.6$, $p<0.01$] exploration [$F(2;48) = 13.0$, $p<0.01$], and the higher motivation of DAT KD mice [$F(2;53) = 5.0$, $p<0.01$]. Conclusion - DAT KD mice exhibited a mania-relevant profile across domains. SCH lowered preferences in the IGT to chance while reducing impulsivity in good-performing DAT KD mice. SCH reduced activity, exploration, and motivation of DAT KD and WT mice. Thus, DRD1 antagonism exerted main effects on behavior while attenuating only some mania-like aspects of DAT KD mice.

Disclosures: **M.B. Milienne-Petiot:** A. Employment/Salary (full or part-time):; UCSD. **M.A. Geyer:** A. Employment/Salary (full or part-time):; UCSD. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Intracellular Therapeutics, Johnson & Johnson, NIDA, NIMH, U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); San Diego Instruments. F. Consulting Fees (e.g., advisory boards); Abbott, Dart, Lundbeck, Neurocrine, Omeros, Otsuka, Sunovion. **J.W. Young:** A. Employment/Salary (full or part-time):; UCSD. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Cerca, Omeros, Lundbeck Ltd, NIMH, U.S. Veteran's Administration

VISN 22 Mental Illness Research, Education, and Clinical Center. F. Consulting Fees (e.g., advisory boards); Amgen, Arena Pharmaceuticals.

Poster

355. Hippocampal Circuits in Fear and Anxiety

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 355.27/CC44

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

CFI

Title: Non-hippocampal memory systems contributing to overtrained context fear memory

Authors: *E. H. SHEPHERD, C. CARTER, H. LEHMANN;
Psychology, Trent Univ., Peterborough, ON, Canada

Abstract: Damage to the hippocampus (HPC) typically causes retrograde amnesia for contextual fear conditioning in rodents. Distributed contextual fear conditioning or overtraining, however, can mitigate the retrograde amnesic effects of extensive HPC damage, suggesting that overtraining can make a context memory become HPC independent. The aim of this study was to determine the structures within the non-HPC memory system supporting this newly HPC-independent memory. Specifically, we examined whether the perirhinal cortex (PRH) and anterior cingulate cortex (ACC), two regions previously associated with context fear memory, are critical components of the non-HPC system supporting overtrained context fear memory. Two experimental approaches were taken: 1) immediate early gene expression assessment in rats tested following contextual fear overtraining and 2) examination of the retrograde amnesic effects of combined HPC+PRH or HPC+ACC damage on overtrained context fear memory. The rats either received 10 context-shock pairings in a single 30-min session (Standard condition) or 10 3-min sessions distributed across five days, each involving one context-shock pairing (Overtraining condition). Thus, the amount of context exposure and the number context-shock pairing were identical in both conditions. Replicating previous findings, lesions of the HPC caused retrograde amnesia in the Standard but not the Overtrained condition, suggesting that overtraining indeed made context fear memory become HPC independent. Expression of the immediate early gene zif268 was found to be significantly greater in the posterior region of the PRH of Overtrained rats compared to Standard condition rats. In addition, the combined HPC+PRH, but not the HPC+ACC lesions impaired the overtrained memory. Therefore, these findings suggest that the PRH, and not the ACC, critically supports a contextual fear memory that has become HPC independent because of overtraining.

Disclosures: E.H. Shepherd: None. **C. Carter:** None. **H. Lehmann:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.01/CC45

Topic: F.02. Animal Cognition and Behavior

Support: R01MH096274

IMHRO Rising Star Award

Title: Hippocampal-prefrontal theta-gamma coupling increases with difficulty in a spatial working memory task

Authors: *M. TAMURA[1,3], T. J. SPELLMAN[1], A. M. ROSEN[1], J. A. GOGOS[2], J. A. GORDON[1];
[1]Dept. of Psychiatry, [2]Dept. of Neurosci., Columbia Univ., New York, NY; [3]Mitsubishi Tanabe Pharma Corp., Yokohama, Japan

Abstract: Cross-frequency coupling contributes to the hierarchy of brain rhythms and is apparent during a variety of cognitive functions. In particular, the amplitude of gamma-frequency (30-120 Hz) oscillations can vary with the phase of theta-frequency (4-12 Hz) oscillations. Theta-gamma coupling can even occur across brain regions. However, little is known about whether and how it contributes to spatial working memory. We examined long-range theta-gamma coupling during performance of a delayed-non-match-to-sample T-maze task. Local field potentials were recorded from wild-type mice (n=9) and mice heterozygous for a deletion of Zdhhc8 (Zdhhc8+/- mice; n=8), a gene within the 22q11.2 microdeletion region that contributes to cognitive dysfunction. Zdhhc8+/- mice showed enhanced vHPC theta-mPFC slow gamma (30-70 Hz) coupling only when the mice successfully performed a spatial working memory task ($p=0.04$), whereas vHPC theta-mPFC fast gamma (80-120 Hz) coupling was not affected by Zdhhc8 haploinsufficiency ($p=0.14$). Theta-gamma coupling strength was also increased by introducing a long delay ($p = 0.04$), or by optogenetically interfering with encoding in wild type mice ($p = 0.02$), two manipulations that increase task difficulty. Finally, single unit analysis revealed that hippocampal theta-prefrontal slow gamma coupling was associated with increased neural synchrony within the mPFC ($r2 = 0.46$, $p = 0.0022$). These findings suggest that enhancement of theta-slow gamma coupling between vHPC and mPFC reflects a compensatory mechanism to maintain spatial working memory performance in the setting of increased difficulty.

Disclosures: **M. Tamura:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Corporation. **T.J. Spellman:** None. **A.M. Rosen:** None. **J.A. Gogos:** None. **J.A. Gordon:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.02/CC46

Topic: F.02. Animal Cognition and Behavior

Support: NIH Predoctoral NRSA F31MH102041

NIMH R01 MH096274

Irma Hirschl Trust

Title: Reciprocal thalamo-prefrontal and prefronto-thalamic projections support overlapping and dissociable spatial working memory processes

Authors: *S. S. BOLKAN[1], S. PARNAUDEAU[1], T. SPELLMAN[1], A. CLARK[1], J. A. GORDON[2], C. KELLENDONK[3];
[1]Neurobio. and Behavior, [2]Dept. of Psychiatry, [3]Dept. of Psychiatry and Pharmacol., Columbia Univ., New York, NY

Abstract: Working memory impairment is a core cognitive symptom of schizophrenia, yet the neural mechanisms underlying working memory remain unclear. Functional imaging studies in patients and healthy subjects, and neurophysiological and lesion studies in model organisms have implicated activity in the mediodorsal thalamus (MD) and prefrontal cortex (PFC) as supporting working memory. However, given the dense, reciprocal connectivity between the MD and multiple PFC subregions, it is unclear (1) whether direct thalamo-prefrontal input to particular PFC subregions may support working memory, (2) whether reciprocal prefronto-thalamic projections may also support working memory, and (3) whether activity in these projections support the processes of working memory encoding, maintenance, or retrieval. To investigate the role of activity in these connections in a temporally precise and reversible manner, we employed the light-driven proton pump eArch3.0 to achieve projection-specific optogenetic inhibition in mice performing a delayed non-match to sample (DNMS) T-maze test of spatial working memory. We find that post-acquisition inhibition of MD-to-mPFC, but not MD-to-OFC, projections impairs behavioral performance when working memory length is increased from 10s to 60s. Inhibition of reciprocal mPFC-to-MD projections similarly caused a delay-dependent impairment in behavioral performance. Interestingly, while temporally precise Sample (encoding) phase inhibition of either projection did not impair behavioral performance, inhibiting activity during the Delay (maintenance) phase of the task lead to robust behavioral impairments with MD-to-mPFC inhibition (RM ANOVA, VirusXLight $p<0.05$) and trend-level impairments with mPFC-to-MD inhibition (RM ANOVA, VirusXLight $p=0.073$). Strikingly, only mPFC-to-MD inhibition during the Choice (retrieval) phase impaired behavioral performance during of the task (RM ANOVA, VirusXLight $p<0.05$). These findings suggest reciprocal thalamo-prefrontal

activity supports the process of working memory maintenance while direct prefronto-thalamic activity supports the process of working memory retrieval and action selection. In an effort to reveal physiological mechanisms supporting these processes, we have obtained simultaneous *in vivo* recordings of dorsal hippocampal and MD local field potentials and mPFC single-units in mice (9 eArch3.0, 9 EYFP) receiving task phase specific MD-to-mPFC inhibition while performing the DNMS T-maze.

Disclosures: **S.S. Bolkan:** None. **S. Parnaudeau:** None. **T. Spellman:** None. **A. Clark:** None. **J.A. Gordon:** None. **C. Kellendonk:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.03/CC47

Topic: F.02. Animal Cognition and Behavior

Support: BBSRC Grant BB/L02134X/1

Title: Contribution of nucleus reuniens of the thalamus to associative recognition memory

Authors: ***G. R. BARKER**[1], E. C. WARBURTON[2];
[2]Physiol. & Pharmacol., [1]Univ. of Bristol, Bristol, United Kingdom

Abstract: Associative recognition memory, the ability to associate an object with a location or position in a sequence, requires interactions between the perirhinal cortex, hippocampus and medial prefrontal cortex (Barker et al 07, Barker & Warburton 11). The nucleues reuniens of the thalamus is reciprocally connected with all 3 brain regions in the associative recognition memory network (McKenna & Vertes 04, Vertes et al 06), and is therefore well placed, anatomically, to be engaged in associative recognition memory formation. Therefore this study aimed to assess the nucleus reuniens contribution to associative recognition memory formation. Two cohorts of male Lister hooded rats were used. The first cohort received either lesions of the midline thalamic nuclei or sham lesions. Associative recognition memory was tested using the object-in-place, temporal order and temporal location tasks, with either a short (5min) or a long delay (3h), non-associative recognition memory was assessed at a long delay (3h) using the object recognition and object location tasks. The second cohort of rats were implanted with guide cannula targeted at the nucleus reuniens. Associative recognition memory performance was assessed using the object-in-place task with memory performance assessed with either a short or a long delay. Intra-cerebral infusions of a number of selective receptor agonists and antagonists into the nucleus reuniens occurred either before the sample phase or before the test phase in order to disrupt different stages of memory processing. Lesions of the midline thalamic nuclei resulted in a delay dependent deficit in all three associative recognition memory tasks tested,

thus performance at the short delay was not altered, while at the long delay lesioned animals showed a significant deficit in memory performance compared to sham animals. Performance in the non-associative recognition memory tasks was not altered. Inactivation of the nucleus reuniens via infusion of muscimol before the sample phase resulted in a delay dependent impairment in associative recognition memory performance, in addition inactivation of the nucleus reuniens before the test phase impaired retrieval. Inhibition of either muscarinic or nicotinic cholinergic receptors but not NMDA receptors in the nucleus reuniens before the sample phase impaired associative recognition memory performance. Therefore these results demonstrate that the nucleus reuniens plays a critical role in long term associative recognition memory and in addition identifies the importance of cholinergic neurotransmission in the nucleus reuniens.

Disclosures: G.R. Barker: None. **E.C. Warburton:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.04/CC48

Topic: F.02. Animal Cognition and Behavior

Support: UNH New Ventures

Title: The influence of decision-making in conditional responding

Authors: *M. J. FRANCOEUR, B. A. WORMWOOD, R. L. A. MILLER, D. C. CHASE, H. D. ROBERTSON, A. G. DRAKE, K. C. ERICKSON, E. S. JALBERT, C. E. WANTE, B. M. GIBSON, R. G. MAIR;
Psychology, Univ. of New Hampshire, Durham, NH

Abstract: Medial prefrontal cortex (mPFC) supports decision-making functions required for delayed nonmatching-to-position (DNMTP). To understand these functions we compared the responses of mPFC neurons in rats performing two tasks: DNMTP and a serial lever pressing (SLP) task in which rats perform all the actions associated with DNMTP without making a choice between response alternatives. Both tasks are trained in octagonal arenas (61 cm in diameter) with four retractable levers located 90° apart. Drinking spouts (for delivery of water reinforcement) and panel lights (to signal reinforcement) are located above each lever. Isolated activity was recorded from 900 neurons in 6 rats performing the DNMTP task. Significant behavioral correlations defined by peri-event timed histogram (PETH) responses beyond the 99% confidence interval were observed for 288 neurons (32%). PETH analyses revealed 10 distinct response types that accounted for 273 (95%) of behaviorally correlated cells: 267 with a unique type and 6 with a combination. Response types were related to initiation, action, outcome,

and memory delays. We are currently studying mPFC activity in rats performing the SLP task. Here levers are extended one at a time, following the pattern used for DNMTP training. Trials begin with a randomly selected base lever extending. This retracts with pressed and the lever 90° to the left or right is extended (equivalent to the DNMTP sample response). This retracts when pressed and is followed by delivery of water reinforcement in the spout immediately above. The base lever for the trial is again extended after a 3 s delay. This retracts when pressed (equivalent to the DNMTP delay response) after which a lever 90° to the left or right is extended. On 70% of trials this is opposite the "sample lever" and retracts when pressed followed by reinforcement (equivalent to a correct DNMTP choice). On 30% of trials, this is the same as the "sample lever" and retracts without reinforcement when pressed (equivalent to an incorrect DNMTP choice). This distribution of reinforced and unreinforced responses models 70% correct criterion responding in the earlier DNMTP recording study. Our results to date confirm the importance of decision making in driving both the level of activity and event-related coding properties of mPFC neurons.

Disclosures: **M.J. Francoeur:** None. **B.A. Wormwood:** None. **R.L.A. Miller:** None. **D.C. Chase:** None. **H.D. Robertson:** None. **A.G. Drake:** None. **K.C. Erickson:** None. **E.S. Jalbert:** None. **C.E. Wante:** None. **B.M. Gibson:** None. **R.G. Mair:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.05/CC49

Topic: F.02. Animal Cognition and Behavior

Support: UNH New Ventures

Title: More than just a relay: The role of medial thalamus in flexible, goal-directed behavior

Authors: R. L. A. MILLER[1], E. B. SMEDLEY[1], M. J. FRANCOEUR[1], B. A. WORMWOOD[1], D. C. CHASE[1], D. MINUKHIN[1], C. J. THERIAULT[1], T. N. KAZAN[1], B. M. GIBSON[1], *R. G. MAIR[2];
[1]Univ. of New Hampshire, Durham, NH; [2]Psychology, Univ. New Hampshire, Durham, NH

Abstract: Convergent evidence suggests that beyond its role as a relay, medial thalamus coordinates the activity of prefrontal cortex (PFC) with distributed neural networks that support intentional responding. The mediodorsal nucleus (MD) is the primary source of specific thalamo-cortical projections to middle layers of PFC while midline and rostral intralaminar nuclei (IL) provide modulatory inputs to deep and superficial layers of PFC and to anatomically-related areas of the basal ganglia and hippocampus. Lesions damaging both MD and IL impair flexible, goal-directed behavior exemplified by delayed non-matching to position tasks (DNMTP). To

elucidate the influence of MD and IL on prefrontal function we recorded the activity of neurons in these nuclei in rats performing a dynamic DNMTP task previously used to characterize medial PFC neuronal activity. Activity was recorded from 8 rats with tetrode arrays that were advanced incrementally through thalamus across 40 to 60 recording sessions. Results were analyzed offline to identify signals from isolated neurons and to correlate activity with specific behavioral events as rasters and peri-event time histograms (PETH) and with spatial location as place fields. Significant behavioral correlations were defined by PETH responses beyond the 99% confidence interval. PETH analyses revealed event-related responses in thalamus consistent with the broad contour of prefrontal response types, with subtle differences in timing. We did not observe evidence of spatially-restricted event-related response observed in PFC. Our results provide evidence that MD and IL have distinct effects on PFC activity.

Disclosures: **R.L.A. Miller:** None. **E.B. Smedley:** None. **M.J. Francoeur:** None. **B.A. Wormwood:** None. **D.C. Chase:** None. **D. Minukhin:** None. **C.J. Theriault:** None. **T.N. Kazan:** None. **B.M. Gibson:** None. **R.G. Mair:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.06/CC50

Topic: F.02. Animal Cognition and Behavior

Support: UNH New Ventures

Title: Medial thalamic modulation of prefrontal cortex: Effects of temporary inactivation

Authors: *B. A. WORMWOOD, M. J. FRANCOEUR, R. L. A. MILLER, D. C. CHASE, E. K. BRASLEY, C. R. LEHET, J. C. MCKEE, A. C. AASEN, B. M. GIBSON, R. G. MAIR; Univ. of New Hampshire, Durham, NH

Abstract: Medial thalamus has reciprocal connections with prefrontal cortex (PFC) that are thought to coordinate its activity with other areas of the telencephalon that support flexible goal-directed behavior. The mediodorsal nucleus is the primary source of specific thalamo-cortical projections to middle layers of PFC while midline and intralaminar nuclei provide modulatory inputs to deep and superficial layers of PFC and to anatomically-related areas of the basal ganglia and hippocampus. Each of these nuclei receive direct cortico-thalamic and indirect cortico- striato-pallidal input from PFC. Medial thalamic lesions impair flexible goal-directed behavior, exemplified by delayed non-matching to position tasks (DNMTP). To elucidate the influence of medial thalamus on PFC function we examined the effects of temporary thalamic inactivation on the response properties of prefrontal neurons in rats performing a dynamic DNMTP task. Cellular activity was recorded throughout medial PFC using a drivable array of

tetrodes. Electrophysiological recordings were analyzed offline to identify signals from isolated neurons and to correlate activity with specific behavioral events as rasters and peri-event time histograms (PETH). We also analyzed cellular activity relative to spatial context using video-tracking data to assess the influence of the animal's location on cell firing rates. Once cells with significant behavioral correlations (PETH responses beyond 99% confidence interval) were identified (day 1), the tetrode array was left in place for two more sessions. On the next day (day 2), central thalamus was unilaterally inactivated using microinjections of the GABAA agonist muscimol. On day 3, activity in the same location was recorded again without thalamic inactivation. We used doses of muscimol previously shown to impair delayed matching to position when injected bilaterally at the same site. Unilateral inactivation had no significant effect on DNMTP performance. Examination of waveforms, inter-spike-interval histograms, cluster analyses, and event related activity (on days 1 and 3) confirmed the identity of single neurons across the three days. Day 2, inactivation was consistently associated with disruption of spatial and event-related activity of prefrontal neurons. Some neurons exhibited increased and others and decreased levels of activity, suggesting both inhibitory and excitatory modulation PFC by thalamus. These data indicate a critical role for medial thalamus in shaping the activity of cortical neurons in relation to actions, outcomes, and context.

Disclosures: **B.A. Wormwood:** None. **M.J. Francoeur:** None. **R.L.A. Miller:** None. **D.C. Chase:** None. **E.K. Brasley:** None. **C.R. Lehet:** None. **J.C. McKee:** None. **A.C. Aasen:** None. **B.M. Gibson:** None. **R.G. Mair:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.07/CC51

Topic: F.02. Animal Cognition and Behavior

Support: MRC Senior non-Clinical Fellowship (MDH)

Title: Signatures of inference-by-sampling in the prefrontal cortex of rule-learning rats

Authors: A. SINGH[1], A. PEYRACHE[2], *M. D. HUMPHRIES[1];
[1]Univ. of Manchester, Manchester, United Kingdom; [2]The Neurosci. Institute, Sch. of Med. and Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The inference-by-sampling hypothesis proposes that neural population activity at each point in time represents a sample from an underlying probability distribution. One key prediction is that the distribution during ``spontaneous'' activity (representing the prior) and during evoked activity (representing the posterior) converge over repeated experience. Just such a convergence has been observed in small populations from ferret V1 over development (Berkes et al 2011,

Science, 331: 83-87). Unknown is the extent to which this hypothesis is a general computational principle for cortex: whether it can be observed during learning, or in higher-order cortices, or during ongoing behaviour. To address all these issues, we analysed population activity from the prefrontal cortex of rats learning rules in a Y-maze task. We focussed on sessions where the animal reached the learning criterion mid-session, allowing us to compare activity before and after learning, and compare activity during sleep pre- and post-session. Population activity in each time bin was characterised as a binary vector (or "word") of active and inactive neurons. Our hypothesis was that the "spontaneous" activity in slow-wave sleep (SWS), in the absence of task-related stimuli and behaviour, constitutes the prior distribution. We find that the distributions of words were highly conserved across all vigilance states (SWS pre- and post-session and during task behaviour), consistent with our interpretation of SWS activity as a "prior". Crucially, the task-evoked distribution after learning was more similar to the distribution in post-session than in pre-session SWS. This increase in similarity only occurred for rewarded trials, not for unrewarded trials. Together, these results are consistent with the convergence of the posterior and prior distributions over learning. The samples from the distribution could also be directly linked to behavioural choice on each trial. The population activity vectors that most changed frequency over the task were also strongly predictive of trial outcome. These updated vectors tended to occur only in maze locations relevant to the currently learnt rule. Our results show that signatures of inference-by-sampling can be observed over the course of learning, in higher-order cortices, and directly related to behavioural choice. Consequently, inference-by-sampling is a potential general computational principle of cortex.

Disclosures: **A. Singh:** None. **A. Peyrache:** None. **M.D. Humphries:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.08/CC52

Topic: F.02. Animal Cognition and Behavior

Support: PM92-0166

BFI2000-0418

BFI2003-09581

BFU2006-12964

BFU2009-14705

Title: Prefrontal cortex afferents to the anterior temporal lobe in the Macaca fascicularis monkey

Authors: *R. INSAUSTI[1], A. MOHEDANO-MORIANO[2], M. MARCOS[2], E. ARTACHO[2], M. ARROYO[2], M. MUNOZ-LOPEZ[2];
[2]Human Neuroanatomy Lab., [1]Univ. of Castilla-La Mancha, Albacete, Spain

Abstract: The anatomical organization of the lateral prefrontal cortex (LPFC) afferents to the anterior part of the temporal lobe (ATL) still needs to be clarified. The LPFC has two subdivisions, dorsal (dLPFC) and ventral (vLPFC) which have been linked to cognitive processes. The ATL includes several different cortical areas, namely temporal polar cortex and rostral parts of perirhinal, inferotemporal and anterior tip of the superior temporal gyrus cortices. Multiple sensory modalities converge in the ATL. All of them (except rostral inferotemporal and superior temporal gyrus cortices) are components of the medial temporal lobe, critical for long-term memory processing. We studied the LPFC connections with the ATL by placing retrograde tracer injections into the ATL: temporal polar (n=3), perirhinal (areas 35 and 36, n=6) and inferotemporal cortices (area TE, n=5), plus one additional deposit in the posterior parahippocampal cortex (area TF, n=1). Anterograde tracer deposits into the dLPFC (A9 and A46, n=2) and vLPFC (A46v, n=2) and orbitofrontal cortex (OFC, n=2) were placed for confirmation of those projections. Results showed that vLPFC displays a moderate projection to rostral area TE and dorsomedial portion of temporal polar cortex; in contrast, the dLPFC connections with the ATL were weak. By comparison, OFC and medial frontal cortices (MFC) showed dense connectivity with the ATL, namely A13 with temporopolar and perirhinal cortices. All areas of MFC projected to temporopolar cortex, albeit with a lower intensity. The functional significance of such paucity of LPFC afferents is unknown.

Disclosures: R. Insausti: None. **A. Mohedano-Moriano:** None. **M. Marcos:** None. **E. Artacho:** None. **M. Arroyo:** None. **M. Munoz-Lopez:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.09/CC53

Topic: F.02. Animal Cognition and Behavior

Support: IRP, NIMH, NIH, HHS

Title: The effect of rhinal cortex-orbitofrontal disconnection on recognition memory in monkeys: comparison of aspiration lesions and reversible silencing with DREADDs

Authors: *M. A. ELDRIDGE, E. C. MASSEAU, W. LERCHNER, J. M. FREDERICKS, R. C. SAUNDERS, B. J. RICHMOND;
Lab. of Neuropsychology, NIMH, Bethesda, MD

Abstract: Introduction It has been proposed that a circuit that includes rhinal cortex (Rh) and orbitofrontal cortex (OFC) among its components subserves object recognition memory. To test this hypothesis, we produced a functional disconnection between these structures by removing the Rh in one hemisphere, and removing the OFC (n = 4), or reversibly silencing OFC using a DREADD (Designer Receptor Activated by Designer Drug) (n = 2), in the contralateral hemisphere. The performance of both groups was compared to that of controls on a test of familiarity-based recognition. Methods: Task In each trial the monkey had to indicate whether the stimulus displayed was presented for the first or second time within the testing session by releasing a lever in one of two intervals: either immediately, when a stimulus was first presented (first interval) or wait until a small central target changed from red to green (second interval) if it was the second appearance of a stimulus. Stimuli were repeated once within the session, with the interval between stimulus presentations within a session varying between 0 to 128 stimuli. The stimulus set consisted of 6000 images. No stimulus was reused within a 30-day period. Methods: DREADD Two monkeys were injected with a modified lentiviral vector expressing a Gi-coupled receptor, hM4Di, in the OFC opposite a Rh removal. When activated by systemically delivered clozapine-N-oxide (CNO), hM4Di induces neuronal silencing. Results Three control monkeys reliably differentiated between the first and second presentations of a stimulus. In contrast, the performance of the monkeys with crossed Rh-OFC lesions was significantly worse than controls at all retention intervals tested. In the two monkeys injected with a DREADD in the OFC opposite a Rh removal, when activated by systemically delivered clozapine-N-oxide (CNO), performance on the recognition memory task was only mildly impaired compared to sessions without CNO administration. Discussion This pattern of results indicates that: 1) An intact rhinal-orbitofrontal connection may only be necessary during the learning of the serial recognition test used here, 2) the volume of OFC tissue inactivated by the DREADD may have been insufficient to replicate the effect of the permanent removal, and/or 3) the sparing of fibers of passage may have been critical.

Disclosures: **M.A. Eldridge:** None. **E.C. Masseau:** None. **W. Lerchner:** None. **J.M. Fredericks:** None. **R.C. Saunders:** None. **B.J. Richmond:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.10/CC54

Topic: F.02. Animal Cognition and Behavior

Support: Canadian Institutes for Health Research

Saskatchewan Health Research Foundation

Natural Sciences and Engineering Research Council of Canada

Title: Neural correlates of odor span capacity in the medial prefrontal cortex of rats

Authors: *L. AN, J. K. CATTON, Q. GREBA, J. G. HOWLAND;
Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Working memory refers to the transient storage of information that was just experienced or retrieved from long-term memory to guide behavior. The contents of working memory are prone to decay but can be stored for longer periods through active rehearsal strategies. Capacity is a component of working memory that is poorly understood, although studies in humans suggest that capacity may critically depend on activity in prefrontal cortex (PFC). In rodents, working memory capacity can be assessed using the odor span task (OST), a sequential non-matching to sample task. Lesions of medial prefrontal cortex (mPFC), which is considered to be functionally homologous to the primate dorsolateral PFC, disrupt performance of the OST; however, the patterns of neural activity in mPFC during the OST are currently unknown. To measure the firing patterns of mPFC neurons in the OST, five male Long-Evans rats were trained on the task and had multichannel probes implanted in the mPFC to record extracellular neural activity during the task. Behavior of the rats was scored manually by an experimenter and broken down into four possible epochs: baseline, stimulus approach, initiation of digging, and reward consumption (following correct responses). For preliminary analyses, we compared the frequency of neural activity occurring 1000 ms around events. Z-scores were computed using baseline firing rates and compared. Ninety-one putative mPFC pyramidal neurons with a mean baseline firing rate at 0.7±0.1 Hz were analyzed. The portion of neurons that increased activity during the task and were selected for further analysis gradually increased from low (span=1-3; 43%) to medium (span=4-6; 45%) and high (span=7-10; 48%) span stages. Activity of the neurons increased when rats approached a scented bowl and activity increased more after approach to a novel bowl than a familiar bowl. Interestingly, neural activity strongly increased when rats dug in a correct bowl that contained reward than when they dug in an incorrect bowl that did not contain reward for medium and high spans. Compared to the medium span test, a potential increase in burst activity between digging and consuming reward was found for high spans, as evidences of increasing the number of bursts per second, the percentage of spikes in bursts, and burst duration. These findings suggest the neural activity of mPFC differs as the function of encoding- and response-related processes. Moreover, the change of burst activity may indicate a possible mechanism of storage and manipulation of information necessary for situations of high odor span capacity.

Disclosures: **L. An:** None. **J.K. Catton:** None. **Q. Greba:** None. **J.G. Howland:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.11/CC55

Topic: F.02. Animal Cognition and Behavior

Support: Friedman Brain Institute of Mount Sinai School of Medicine

Title: Chemogenetic suppression of neural activity in dorsolateral prefrontal cortex impairs spatial working memory in rhesus monkeys

Authors: W. SCHNEBELEN, P. G. F. BROWNING, P. L. CROXSON, P. H. RUDEBECK, S. W. BROOKSHIRE, *M. G. BAXTER;
Dept Neurosci., Mount Sinai Sch. Med., New York, NY

Abstract: The DREADD (Designer Receptors Exclusively Activated by Designer Drugs) methodology allows for expression of an artificial receptor system in an anatomically and/or neurochemically-defined population of neurons. These designer receptors are derived from human muscarinic G-protein coupled receptors, allowing for physiologically relevant manipulations in activity. DREADDs remain quiescent until activated by an otherwise inert pharmacological agent, clozapine-N-oxide (CNO). We placed multiple injections of AAV5 vector containing a construct for the inhibitory hM4Di DREADD receptor, under control of the human synapsin promoter, bilaterally into the dorsolateral prefrontal cortex of male rhesus monkeys. Monkeys performed the delayed response test of spatial working memory in a manual test apparatus. Task performance was unaffected by the surgery or by vehicle injections, but was dramatically impaired by intramuscular injection of CNO. Functional DREADD receptor expression lasted at least a year post-surgery and was verified histologically. The performance of monkeys that had not received DREADD AAV injections was unimpaired by CNO. These initial observations support the effectiveness of this chemogenetic system for neurobiological investigations in macaque monkeys, and open new experimental modalities in this species.

Disclosures: W. Schnebelen: None. **P.G.F. Browning:** None. **P.L. Croxson:** None. **P.H. Rudebeck:** None. **S.W. Brookshire:** None. **M.G. Baxter:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.12/CC56

Topic: F.02. Animal Cognition and Behavior

Support: NEI grant R01EY017077

NINDS grant T32NS073553

NIMH grant F31MH104012

Title: Functional specialization of areas along the anterior-posterior axis of the primate prefrontal cortex prior to training in a task

Authors: *M. RILEY, X. QI, C. CONSTANTINIDIS;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The prefrontal cortex (PFC) is critical for working memory and executive function. Functional specialization of areas along the anterior-posterior PFC has been speculated but little evidence exists about the functional properties of neurons in these areas. To address this question we segmented the dorsal PFC into 3 regions: posterior-dorsal (area 8A of Petrides and Pandya), mid-dorsal (area 8B and area 9/46), and anterior-dorsal (area 9 and area 46). We contrasted the responsiveness and selectivity of neurons in these regions with the posterior ventral region (area 45). Three stimulus sets were used to evaluate selectivity: a spatial set consisting of 9 white squares arranged in a 3x3 grid of 10° eccentricity between stimuli; a feature set consisting of 8 white geometric shapes matched for total area; and a color set consisting of 8 equiluminant color squares. A total of 1878 neurons were recorded in 6 monkeys passively viewing these stimuli, prior to training in a working memory task. There was no significant difference in the total percentage of neurons that responded to the stimuli between the anterior, mid and posterior dorsal area (chi-square test, $p>0.5$), though the percentage of neurons responding to stimuli in the ventral posterior area was significantly lower than the dorsal areas (posterior-dorsal: 44%; mid-dorsal: 41%; anterior-dorsal 35%; posterior-ventral 20%; chi-square test, $p<0.05$). On the other hand, selectivity for stimuli was highest for the posterior-dorsal area and generally declined along the anterior-posterior axis. When quantified with a selectivity index expressed as (Max-Min)/(Max+Min), significant differences were present for the spatial, feature, and color selectivity values across areas (spatial: posterior 0.693, mid 0.577, anterior 0.566; feature: posterior 0.492, mid 0.385, anterior 0.411; color: posterior 0.420, mid 0.267, anterior 0.256; 1-way ANOVA, $p<0.05$, for each stimulus set). In addition, firing rates during the presentation of the stimuli were significantly higher for the posterior-dorsal area (posterior 14.5 spikes/s, mid 9.9 spikes/s, anterior 10.3 spikes/s; 1-way ANOVA, $p<0.05$). These differences were only seen during the display of the stimuli; activity during the baseline fixation period did not significantly differ between areas (1-way ANOVA, $p>0.07$). Our results provide neurophysiological evidence for a rostral-caudal gradient of stimulus selectivity through the PFC, suggesting that posterior areas are selective for stimuli even when these are not relevant for execution of a task whereas anterior areas may acquire selectivity as a result of task performance.

Disclosures: **M. Riley:** None. **X. Qi:** None. **C. Constantinidis:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.13/CC57

Topic: F.02. Animal Cognition and Behavior

Support: PHS Pioneer Award DP1AG047744-01

Title: Physiological differences between the primary visual cortex and dorsolateral prefrontal cortex in primate

Authors: *S. YANG, M. WANG, C. D. PASPALAS, M. ALTMAN, L. E. JIN, V. GALVIN, A. F. T. ARNSTEN, J. A. MAZER;
Dept. of Neurobio., Yale Univ., New Haven, CT

Abstract: Very little is known about the molecular modulation of neuronal firing patterns in the primate primary visual cortex (V1). In classic synapses, cAMP-protein kinase A (PKA) signaling has been shown to increase transmitter release through actions on pre-synaptic vesicles, strengthen post-synaptic mechanisms, and/or increase the uptake of glutamate from the synapse due to increased expression of excitatory amino acid transporters on glia. Immunoelectron microscopy to localize phosphodiesterase PDE4A is consistent with this pattern, where PDE4A is most commonly found in presynaptic glutamate terminals, in peri-synaptic glia, and occasionally in mushroom-shaped spines. In contrast, PDE4A in layer III of the dorsolateral prefrontal cortex (dlPFC) is most evident in long thin spines, anchored near the calcium-containing spine apparatus near HCN channels. In dlPFC, increased cAMP signaling weakens connectivity and reduces the firing of "Delay cells" (neurons that maintain representations of visual space across delay periods) by opening HCN channels on spines. The current research is the first exploration of cAMP-PKA signaling influences on primate V1 neuronal firing for direct comparison to the dlPFC. Single units were recorded from V1 monkeys viewing visual stimuli which are black or white bars presented at and around the receiptive field, while using iontophoresis to deliver minute amounts of charged compounds near the recording site. Results were compared to those from dlPFC recordings from monkeys performing a visual spatial working memory task. In dlPFC, increasing endogenous cAMP levels via iontophoresis of the PDE inhibitor, etazolate, markedly decreased Delay cell firing; this reduction was reversed by simultaneous application of HCN channel blocker, ZD7288. Low doses of ZD7288 alone enhanced the firing of Delay cells, selectively increasing firing for the neurons' preferred direction. In contrast, V1 neurons showed reduced firing with iontophoresis of ZD7288. Etazolate had a mixed influence on V1 neurons, increasing firing in some neurons, having no effect in others, or reducing firing. The reduction in firing of V1 neurons by etazolate was not reversed by ZD7288, indicating a different underlying mechanism (e.g. increasing glutamate transport from the synapse) than the HCN mechanism prevalent in dlPFC. These data show that modulation mechanisms in V1 neurons are fundamentally different from those in dlPFC Delay cells, which may contribute to the resilience of the primary visual cortex in disorders such as Alzheimer's Disease.

Disclosures: S. Yang: None. **M. Wang:** None. **C.D. Paspalas:** None. **M. Altman:** None. **L.E. Jin:** None. **V. Galvin:** None. **A.F.T. Arnsten:** None. **J.A. Mazer:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.14/CC58

Topic: F.02. Animal Cognition and Behavior

Support: 5R01DA026297-04-NIH-NIDA

5R01EY017658-05-NIH-NEI

Title: Single unit and network dynamics within prefrontal cortex and the basal ganglia during working memory maintenance and updating

Authors: *D. HUIE, K. GHOSE, C. MARTINEZ-RUBIO, A. PAULK, T. M. HERRINGTON, E. N. ESKANDAR;
Massachusetts Gen. Hosp., Boston, MA

Abstract: Working memory is the capacity to briefly hold and recall information, which is both robustly maintained against distractions but may also be flexibly updated when necessary. While working memory maintenance has primarily been ascribed to prefrontal cortex (PFC), the basal ganglia is thought to play a "gatekeeper" role, allowing certain stimuli to access and change working memory while protecting working memory from distracting input. This gating hypothesis has been encapsulated within abstract and computational models, and received empirical support from studies in healthy patients and those with working memory deficits. However, both single-unit and targeted local field potential (LFP) studies to clarify the role of these structures in working memory are lacking. We describe an ongoing study to analyze single-unit activity as well as LFP changes used to characterize network connectivity within the corticostriatal circuit during working memory function. Specifically, we present the results of microelectrode recordings within dorsolateral PFC and the anterior caudate nucleus in two rhesus macaques, trained to perform two working memory tasks designed to examine working memory updating and maintenance. Through simultaneous recordings in both structures, we tested the role of the corticostriatal network in working memory. Loss of working memory is a devastating feature of many neurodegenerative diseases; a detailed understanding of corticostriatal circuit's role may illuminate new therapeutic targets for cognitive deficits in these conditions.

Disclosures: D. Huie: None. **K. Ghose:** None. **C. Martinez-Rubio:** None. **A. Paulk:** None. **T.M. Herrington:** None. **E.N. Eskandar:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.15/CC59

Topic: F.02. Animal Cognition and Behavior

Support: NIH-NEI - 2R01EY017658-06A1

1P50MH086400-05

Title: Early phases of learning and novelty encoding by basal forebrain activity correlated with network connectivity to the dorsolateral prefrontal cortex

Authors: *C. MARTINEZ-RUBIO[1], A. PAULK[1], D. SIERRA-MERCADO[2], E. MCDONALD[1], E. ESKANDAR[1];
[1]Neurosurg., Massachusetts Gen. Hosp., Boston, MA; [2]Anat. and neurobiology, Univ. of Puerto Rico, Med. Sci., San Juan, Puerto Rico

Abstract: Stimuli recognition, novelty, and reward are critical factors for learning and consolidating new associations. Brain areas that appear to be major candidates responsible for learning novel associations are the basal forebrain and the prefrontal cortex. This relationship between the basal forebrain and learning is especially evident in patients with dementia and Alzheimer's disease, when degeneration of neurons in the Nucleus Basalis of Meynert (NBM) results in impaired learning and memory. However, the neural signatures via which the NBM is involved in cognitive function, and, in particular, learning, remain unclear. We hypothesized that the firing rate of different populations of neurons in the NBM will differentiate between epochs of an associative learning task. Furthermore, we hypothesize that network connectivity between the NBM and the dorsolateral prefrontal cortex (DLPFC) will change depending on the learning state. To test this idea, we recorded neuronal activity simultaneously from both the NBM and the DLPFC in behaving non-human primates (NHP) performing an associative learning task. During this task, the animal was required to learn, by trial and error, to associate a visual image with a specific direction of eye movement. We recorded from a total of 422 units, with 223 units in the DLPFC and 199 units in the vicinity of the NBM. Our results show that, as the NHPs are exposed to novel stimuli, the firing rate of NBM neurons would often either increase or decrease significantly when the stimulus is novel. Yet, with additional exposures to the stimulus, this modulation reduced significantly to the point that the responses disappeared around trial 50. On the other hand, the DLPFC would modulate neuronal responses depending on what image was presented or would begin to respond later in the trial. We tested whether this was actually due to learning or novelty by presenting the same stimulus later in the experiment but with changed association to eye movement. We found that the NBM neurons would change their firing rates by small amounts early on in the block of training, but this modulation would again disappear as the experiment continued. Contrary to the single unit data, however, we found that high frequency bands (gamma) in the local field potential (LFP) at the NBM and basalis increased as the animal learned. In addition, coherence between the two brain regions increased during the same epochs, speaking to differences in the network dynamics which can be separable from the single-neuron activity. In sum, we found that both the DLPFC and NBM exhibit changes in learning and novelty, which may have major implications for clinical interventions.

Disclosures: **C. martinez-Rubio:** None. **A. Paulk:** None. **D. Sierra-Mercado:** None. **E. McDonald:** None. **E. Eskandar:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.16/CC60

Topic: F.02. Animal Cognition and Behavior

Support: GACR 14-03627S

Title: Effect of medial prefrontal, anterior cingulate, and retrosplenial cortex lesion on cognitive coordination and flexibility using Carousel maze in rats

Authors: ***J. SVOBODA**[1], I. VOJTECHOVA[1,2], A. STANKOVA[1,2], A. STUCHLIK[1,2]; [1]Inst. of Physiol. CAS, Prague 4, Czech Republic; [2]Natl. Inst. of Mental Hlth., Klecany, Czech Republic

Abstract: Cognitive coordination involves the ability to segregate conflicting stimuli into coherent, but mutually discordant subsets, while cognitive flexibility allows for rapid change of a strategy if the old one is found ineffective. Carousel maze provides a tool to investigate both coordination and flexibility in spatial domain in rodents. On a continuously rotating circular arena, rats have to avoid a place hidden in either the stationary room or the rotating arena frame. They have to selectively attend to cues from the relevant frame and ignore the others. This process is considered to reflect cognitive coordination. Furthermore, in well-trained animals, we can explore two types of flexibility: 1) by interchanging the relevant frame of reference (set-shifting task), or 2) by relocating the prohibited sector within the same reference frame (reversal task). We assessed a role of medial prefrontal (mPFC), anterior cingulate (ACC), and retrosplenial (RSC) cortex in mediating the above mentioned processes by excitotoxic lesions of the corresponding site in male Long-Evans rats. Results showed that particularly RSC, but not mPFC or ACC, contributes to coordination and flexibility and thus complements the role of hippocampus which has been traditionally considered crucial for navigation in Carousel maze. These results may provide a new link between impaired cognitive flexibility in schizophrenia and its substrate in neocortex. The study was supported by GACR 14-03627S.

Disclosures: **J. Svoboda:** None. **I. Vojtechova:** None. **A. Stankova:** None. **A. Stuchlik:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.17/CC61

Topic: F.02. Animal Cognition and Behavior

Support: European Union FET open program: Enlightenment, grant nr: 284801

Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) Earth and Life Sciences Open program grant: Lifetime of Memories

Title: State-dependent network response to optogenetic stimulation in prefrontal cortex

Authors: *Z. NAVRATILOVA, H. O. CABRAL, F. P. BATTAGLIA;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: We were interested in how local optogenetic stimulation affects larger neural networks, and hypothesized that this effect varies according to the sleep/wake state of the animal. To study these effects, we injected a viral vector containing channelrhodopsin (mChR2) with an alpha-CamKII promoter in medial prefrontal cortex (mPFC), and subsequently implanted an array of 20 independently movable tetrodes along with one or two fibers for optical stimulation. We measured the local field potential and spiking activity during stimulation at these 20 sites distributed up to 1 mm away from the stimulation site. We observed various multi-unit spiking responses (MUA) to the stimulation. Some tetrodes displayed a continuous firing rate increase throughout the stimulation (of up to 200ms), while some showed a transient increase in firing rate at the onset of stimulation, which lasted only about 30ms. Many tetrodes distant from the stimulation site showed a delayed transient increase in firing rate. At the offset of the stimulation, many tetrodes showed a reduction in firing rate to below the non-stimulation average rate. The gamma frequency (50-100 Hz) activity recorded on each tetrode was also enhanced during stimulation, and more so on the tetrodes with high MUA responses. More interestingly, the spiking and gamma responses depended on the sleep state of the rat. During recording, the rats were sitting quietly in a small box, and so sometimes they were alert and showing awake cortical activity, and other times they were sleeping and showing slow wave oscillations. Tetrodes with the highest and fastest firing rate and gamma responses to the stimulation were responsive regardless of state, but tetrodes with low activation responses only showed those responses during the slow oscillatory state. The slow oscillation consists of “up” and “down” states of cortical spiking activity. These states alternate at a rate of 0.5-1 Hz. Down states are associated with delta (1-4 Hz) waves in the LFP, and up states sometimes contain spindle (10-15 Hz) activity. We observed that during the slow oscillation, the offset of optogenetic stimulation was associated with an increased likelihood of down state onset and delta waves. By contrast, during awake states, neither delta waves nor down states were triggered. We compare stimulation-triggered down states and delta waves to those occurring naturally. We conclude that local optogenetic stimulation affects local neural activity similarly during wake and sleep behavioral states, but during more ordered neural network states, such as non-REM sleep, this stimulation can have wider impacts, such as organizing the occurrence of up and down states.

Disclosures: **Z. Navratilova:** None. **H.O. Cabral:** None. **F.P. Battaglia:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.18/CC62

Topic: F.02. Animal Cognition and Behavior

Support: IBS-R002-G1

National Research Foundation grant 2011-0015618

Title: Physiological characteristics of anterior insular and orbitofrontal cortices in representing uncertain reward

Authors: ***S. JO**[1,2], M. W. JUNG[1,2,3];
[1]Inst. For Basic Sci., Daejeon, Korea, Republic of; [2]Ajou Univ. Sch. of Med., Suwon, Korea, Republic of; [3]Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Humans and animals often choose a safe over a risky option even when the risky option has a higher expected value, indicating that decision making is affected by risk. Anterior insular and orbitofrontal cortex (AIC and OFC, respectively) are known to play important roles in decision making under risk. However, risk-related neural activity has not been investigated in the AIC and it is controversial whether the OFC conveys genuine risk signals. To address these issues, we recorded neural activity in the AIC and OFC of rats responding to five distinct auditory cues predicting reward delivery with different probabilities. Both structures conveyed significant neural signals for reward, value and risk, with value and risk signals conjunctively coded. However, value signals were stronger and appeared earlier in the OFC, and many risk-coding OFC neurons responded only to the cue predicting certain (100%) reward. Also, AIC neurons tended to increase their activity following a negative outcome, which persisted until the next trial, and according to previously expected value. These characteristics might underlie distinct roles of the AIC and OFC in decision making under risk. The OFC might play an important role in encoding certain reward-biased, risk-modulated subjective value, whereas the AIC might convey prolonged disgust and disappointment signals.

Disclosures: **S. Jo:** None. **M.W. Jung:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.19/CC63

Topic: F.02. Animal Cognition and Behavior

Title: Distinct roles of parvalbumin- and somatostatin-positive interneurons in working memory

Authors: *D. KIM[1], H. JEONG[2], J.-W. GHIM[3], S.-H. LEE[2], M. JUNG[1,2,3];
[1]Grad. Sch. of Med. Sci. and Engin., [2]Dept. of Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [3]Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic Sci., Daejeon, Korea, Republic of

Abstract: Working memory refers to the system of temporarily maintaining and manipulating information required to perform complex cognitive tasks. Persistent and sequential neuronal discharges have been observed as potential neural substrates for working memory, and properly tuned excitation and inhibition are thought to be critical for such neuronal activity. Although recent studies have begun to reveal distinct roles of different interneuron subtypes in various regions of the brain, specific roles of different inhibitory interneuron subtypes in working memory remain unclear. We investigated discharge characteristics of two major interneuron subtypes, parvalbumin (PV)- and somatostatin (SOM)-positive neurons, in medial prefrontal cortex (mPFC) of mice performing a spatial working memory task. Wide-spike SOM neurons showed target-dependent activity during the delay period, which was comparable to delay-period activity of putative pyramidal neurons, whereas PV and narrow-spike SOM neurons showed little target-dependent delay-period activity. In addition, PV and narrow-spike SOM neurons, but not putative pyramidal and wide-spike SOM neurons, were markedly and homogeneously suppressed after reward delivery. Our results demonstrate distinct activity patterns of PV and SOM neurons related to working memory and reward processing.

Disclosures: **D. Kim:** None. **H. Jeong:** None. **J. Ghim:** None. **S. Lee:** None. **M. Jung:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.20/CC64

Topic: F.02. Animal Cognition and Behavior

Title: Inactivation of CA1, but not CA3, impairs retrieval of spatial memory

Authors: *J. LEE[1], J. LEE[2], Y. JEONG[1], M. JUNG[1,2];
[1]KAIST, Daejeon, Korea, Republic of; [2]IBS, Daejeon, Korea, Republic of

Abstract: Hippocampus is a crucial brain structure for spatial learning and memory. Despite a long history of intensive investigation, roles of each hippocampal subregion (dentate gyrus, CA3 and CA1) in encoding and retrieval of spatial memory are unclear. In this study, we compared effects of suppressing CA3 or CA1 neural activity on the retrieval of spatial memory using a pharmacogenetic approach. We injected virus carrying *Cre*-dependent *hM4Di* bilaterally into dorsal CA3 and CA1 of *Grik4-Cre* and *CamkII-Cre* mice, respectively. Four weeks after the viral injection, the mice were trained to find a hidden platform in a Morris water maze for 7 days, which reduced the escape latency to < 20 s. The animals were then tested with CNO or vehicle (DMSO) injection (i.p.). CNO-injected *CamkII-Cre* mice were significantly impaired, whereas CNO-injected *Grik4-Cre* mice showed intact performance in escaping to the hidden platform. Our results indicate the importance of intact CA1 neural activity for the retrieval of spatial memory. When CA3 neural activity is suppressed, direct entorhinal-CA1 projections and/or dentate gyus-CA2-CA1 projections might be able to support the memory retrieval process. Alternatively, intact CA1 neural activity might be reinstated from partially suppressed CA3 neural activity based on pattern completion.

Disclosures: **J. Lee:** None. **J. Lee:** None. **Y. Jeong:** None. **M. Jung:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.21/CC65

Topic: F.02. Animal Cognition and Behavior

Support: F32 AA02314

U01AA019967

P50AA10761

R01AA10983

Title: Task-modulated activity in the infralimbic PFC and accumbens shell during goal-directed vs habitual behavior

Authors: *W. B. GLEN, JR[1], J. M. BARKER[2], L. J. CHANDLER[2];
[1]Neurosciences, [2]Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Behavioral adaptability allows for flexible responding to rules and contingencies between reward and outcomes. Goal-directed behavior allows for the acquisition of new action-outcome relationships, allowing for the development of new behavioral patterns to capitalize on current contingencies. The transition from goal-directed behaviors to habitual responding allows for more of an increase in efficiency during the prolonged capitalization of such contingencies.

As habitual behaviors are driven by stimuli rather than action-outcome relationships, this increased efficiency comes at a cost to behavioral adaptability. In the face of changing rules and contingencies, previous habits can become suboptimal or even maladaptive. Many psychiatric disorder are characterized in part by the expression of inappropriate habits, including substance abuse, eating disorders, and obsessive-compulsive disorder. A variety of brain regions have been identified as essential for the development and expression of habitual behavior, including the infralimbic cortex (IL). While much attention has been paid to the striatum, relatively less focus has been given to the infralimbic cortex. In this study, multielectrode array recordings were taken in awake and behaving mice in order to to examine the electrophysiological activity in the IL as well as its downstream target in the striatum, the shell of the nucleus accumbens (NAcS). Recordings were taken during the acquisition of two operant responses for sucrose (10%). These responses were reinforced on two different reinforcement schedules: random interval (RI) and random ratio (RR). As expected, we observed that responding on these RR schedules remained goal-directed, while responding on an RI schedule became habitual over time. as assessed through outcome devaluation. Ongoing analysis of electrophysiological data during the acquisition and expression of both RR and RI schedules will examine the spectral and firing properties of activity within the IL and NAcS during behavior. Results suggest a variety of task-modulated changes in activity dependent on the development of habitual behavior, including increased delta power (1-4hz) during RI during late sessions which was not observed in RI early sessions, or RR late sessions.

Disclosures: **W.B. Glen:** None. **J.M. Barker:** None. **L.J. Chandler:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.22/CC66

Topic: B.08. Synaptic Plasticity

Support: NIDA Intramural Research Program

Title: Identifying functional alterations in neuronal ensembles activated during acquisition of operant learning in rats

Authors: *L. R. WHITAKER, K. B. MCPHERSON, B. L. WARREN, J. M. BOSSERT, Y. SHAHAM, B. T. HOPE;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Learned associations between environmental stimuli and rewards drive goal-directed learning and motivated behavior. These associations are thought to be encoded by specific patterns of sparsely distributed neurons called neuronal ensembles that are selectively activated

by reward-predictive stimuli. The question remains as to how neurons in these ensembles are functionally altered during learning, and which of these changes encode learned associations. The objectives of our study were to identify ensembles of neurons strongly activated during acquisition of operant learning, and then to determine functional alterations specific to these neuronal ensembles. During each training session, rats were allowed to lever press for food pellets in the self-administration chambers for one hour. Rats formed an association between lever pressing and food reward over the course of several days of training. Immunohistochemical analysis indicates induction of Fos expression in ventral medial prefrontal cortex and nucleus accumbens on day 1 of training relative to home cage controls. These regions are critical to the acquisition of goal-directed learning and motivated behavior. Interestingly, Fos expression diminished over the course of training in infralimbic cortex, but remained consistent in prelimbic cortex. In future experiments, we will use Fos-GFP transgenic rats in which strong neuronal activation activates the Fos promoter that drives expression of GFP. Whole cell brain slice electrophysiology will then be used to assess functional alterations in behaviorally activated GFP-labeled neuronal ensembles. This work was supported by NIDA Intramural Research Program.

Disclosures: **L.R. Whitaker:** None. **K.B. McPherson:** None. **B.L. Warren:** None. **J.M. Bossert:** None. **Y. Shaham:** None. **B.T. Hope:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.23/CC67

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1ZIADA000587

Title: Correlates of inferred associations in orbitofrontal neurons observed during sensory preconditioning

Authors: *B. F. SADACCA[1], H. WIED[2], G. SAINI[3], D. NEMIROVSKY[3], G. SCHOENBAUM[3];
[1]Natl. Inst. On Drug Abuse, Baltimore, MD; [2]Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: The orbitofrontal cortex (OFC) has been implicated in a range of functions, but converging evidence points to OFC's role in supporting 'model-based' decision making. Such decisions are characteristically based on the use of inference, where the value of options is calculated on the fly from the animal's broader experience. Our lab has shown that the orbitofrontal cortex was necessary for just such a process through the use of a sensory-

preconditioning task, in which rats learn associations between pairs of cues in the absence of reward, and then learn that one cue of one pair leads to a food reward. Normal rats are able to use information acquired independently in these phases of learning later, to infer the value of the preconditioned cue both to guide behavior and to direct future learning, whereas rats in which the OFC is inactivated cannot. To identify neural correlates of this process, we recorded single-neuron activity in orbitofrontal cortex of 20 rats as they learned a sensory-preconditioning task. During the initial pre-conditioning phase of the task, as rats learned a relationship between pairs of cues in the absence of reward, OFC neurons tracked the association of cue pairs, responding more similarly to cues within an associated pair than to cues which were unpaired. This similarity in responding, while diminished, remained after the subsequent conditioning phase. Specifically, on the final test day, single neuron responses to cues that were paired during pre-conditioning were more similar to each-other than to control cues. . Thus the encoding of the cue pairs established during preconditioning may be the neural basis for OFC's role in inferring the relationship among cues required to perform a sensory preconditioning task and, more broadly, for OFC's role in inferential reasoning.

Disclosures: B.F. Sadacca: None. **H. Wied:** None. **G. Saini:** None. **D. Nemirovsky:** None. **G. Schoenbaum:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.24/CC68

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH105125

NSF Grant IOS0922075

NSF Grant IOS1353173

Title: Lesions of the retrosplenial cortex attenuate context fear conditioning, but not incidental context learning

Authors: *T. P. TODD, N. E. DEANGELI, M. Y. JIANG, D. J. BUCCI;
Psych & Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Contextual fear conditioning can theoretically be supported by two distinct processes: 1) elemental or feature associations, and 2) hierarchical or conjunctive representations (Rudy, 2009; Rudy et al., 2004). In the feature view, individual elements of the context are independently associated with shock. In the conjunctive view, features of the environment are bound together into a configural unit, and the entire representation is then associated with shock.

The conjunctive view gains support from evidence that pre-exposure to the conditioning context alleviates the immediate shock deficit (Fanselow, 2000). With the immediate shock procedure, rats show very little fear to the context, presumably due to an insufficient amount of time to sample the environment. However, pre-exposure alleviates this deficit because it allow for the formation of a cohesive, conjunctive representation of the context, that can then be retrieved by a single feature and subsequently associated with shock. Lesions or temporarily inactivation of the hippocampus disrupt the facilitative effect of context pre-exposure, suggesting the hippocampus has a crucial role in acquiring a conjunctive representation of the context (Rudy, Barrientos, & O'Reilly, 2002). The present experiments examined the role of the retrosplenial cortex (RSC) in contextual fear conditioning. While it has been suggested that neocortical regions support feature and not conjunctive representations, the specific role of the RSC has not been tested. Rats with electrolytic lesions of the RSC were exposed to the conditioning context (Context A) for 2 minutes on 4 consecutive days, while control rats were exposed to Context B. On day 5, all rats were placed in Context A and received a 1 mA, 2 s shock, 7 seconds after placement in the chamber. Rats were tested for contextual fear 24 hours later. Both RSC lesioned and Sham rats showed the context pre-exposure facilitation effect: prior exposure to the context increased fear, relative to rats exposed to a different context. Further, context fear was equivalent between RSC lesioned and Sham rats irrespective of if they were preexposed to Context A or B. In a second experiment, we confirmed that the RSC lesions were efficacious, replicating the finding that pre-training RSC lesions impair context fear learning (e.g., Keene & Bucci, 2008). Our findings suggest that in contrast to the hippocampus, the RSC is not required to form a conjunctive representation of the context. This in turn implies that deficits in contextual fear produce by RSC lesions are likely due to an inability to form associations between context features and shock.

Disclosures: T.P. Todd: None. **N.E. DeAngeli:** None. **M.Y. Jiang:** None. **D.J. Bucci:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.25/CC69

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH105125

NSF Grant IOS0922075

NSF Grant IOS1353173

Title: Chemogenetic silencing of the retrosplenial cortex disrupts retrieval of remote trace fear

Authors: *N. DEANGELI, T. P. TODD, D. J. BUCCI;
Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: The retrosplenial cortex (RSC) is positioned at the interface between primary cortical sensory areas and parahippocampal and hippocampal regions, and is strongly interconnected with parahippocampal regions (Sugar et al., 2011). Studies using delay fear conditioning procedures have demonstrated that RSC is involved in contextual fear memory. For example, permanent lesions (Keene and Bucci, 2008) as well as temporary inactivation of RSC impair the retrieval of contextual fear (Corcoran et al., 2011). In contrast, the existing data suggest that RSC is not critically involved in recalling fear to individual cues (Keene and Bucci, 2008). However, these studies have focused on retrieving recent fear memories, whereas the involvement of RSC in retrieving older (i.e. remote) memories remains largely untested. Indeed, preliminary data from our lab demonstrated that lesions of RSC 28 days after conditioning resulted in attenuated retrieval of cue-specific fear. We sought to extend this research by temporarily inactivating RSC at the time of retrieval instead of permanently lesioning RSC. We examined a trace CS-US relationship, considering recent evidence that the RSC is involved in retrieval of *recent* trace fear memories (Kwapis et al., 2014). The RSC was temporarily inactivated using DREADDS (designer-receptors-exclusively-activated-by-designer- drugs, Armbruster et al., 2007; Urban and Roth, 2014). Rats were infused with a viral vector containing the gene for a synthetic inhibitory G-protein-coupled receptor (hM4Di, Gi) into the RSC (n = 8), or a viral vector containing only the gene for green fluorescent protein (GFP; n = 8). Conditioning consisted of a single 10 min conditioning session in Context A, with three cue-shock pairings (there was a 20 s trace between CS and US). The following day, all rats were exposed to Context A following injection of CNO. Silencing RSC neurons had no effect on the retrieval of context fear. After a 28-day retention interval, rats were again injected with CNO 30 minutes prior to being exposed back to Context A for 20 min, for two consecutive days. The next day, rats were placed in Context B for 20 minutes without CNO, in an effort to reduce any baseline fear prior to the tone retrieval session. Finally, all rats were injected with CNO and retrieval of tone fear memory was tested. Silencing neurons in the RSC attenuated retrieval of fear to the tone, indicating that the RSC is involved in the retrieval of a trace conditioned fear memory originally conditioned 28 days prior. Thus, the contribution of RSC to context and cued-fear is more complex than originally thought; it depends upon the age of the memory, and the CS-US relationship.

Disclosures: **N. Deangeli:** None. **T.P. Todd:** None. **D.J. Bucci:** None.

Poster

356. Learning and Memory: Prefrontal and Retrosplenial Cortex

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 356.26/CC70

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH105125

NSF Grant IOS0922075

NSF Grant IOS1353173

Title: Lesions of retrosplenial cortex have no impact on renewal of extinguished fear, but attenuate context fear conditioning

Authors: *M. JIANG, N. E. DEANGELI, D. J. BUCCI, T. P. TODD;
Dartmouth Col., Hanover, NH

Abstract: The retrosplenial cortex (RSC) contributes to spatial and contextual learning and memory (e.g., Todd & Bucci, 2015). For example, pre-training lesions of RSC impair contextual fear learning, but have no impact on fear to a Pavlovian conditioned stimulus (CS). However, CSs that undergo extinction (i.e., repeatedly presented alone resulting in a decrease of the conditioned response) are often especially dependent upon the context for retrieval. The purpose of the current experiments is to examine the role of RSC in extinction and renewal of conditioned fear. Rats with either sham lesions or electrolytic lesions of RSC were first trained to lever press for food reinforcement in two distinct contexts (Context A and B). Next, a flashing light stimulus was paired with shock in Context A over the course of two, 2-day cycles. On the first day of the cycle, the light was paired with shock 4 times in Context A. On the second day of the cycle, rats were exposed to Context B and allowed to lever press freely for reinforcement. Following conditioning, the CS was extinguished in Context B. Similar to conditioning, extinction occurred over the course of four, 2-day cycles. On the first day of the cycle, the light CS was presented 8 times in Context B. On the second day of the cycle, rats were exposed to Context A and allowed to freely lever press for reinforcement. In the test for renewal, all rats received 4 nonreinforced presentation of the light CS in both Contexts A and B (test order counterbalanced). Lesions of RSC had no impact on the rate of acquisition, or the rate of extinction to the Pavlovian CS. Further, during renewal testing, there was a robust return of fear for both groups; fear to the CS was high in Context A, but low in Context B. To confirm the efficacy of the RSC lesions, following renewal testing there was a single context fear conditioning session in which the context alone was paired with shock 8 times. During this session, sham rats gradually reduced their rate of lever pressing, whereas RSC lesioned rats did not. Our results suggest that the RSC is necessary for some, but not all, forms of contextual learning. Specifically, when the context is required to disambiguate the meaning of the CSs (e.g., renewal) the RSC does not appear to be necessary. However, RSC is necessary to learn direct associations between context and shock.

Disclosures: **M. Jiang:** None. **N.E. DeAngeli:** None. **D.J. Bucci:** None. **T.P. Todd:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.01/CC71

Topic: F.02. Animal Cognition and Behavior

Support: EU FP7 NEUROSEEKER

Title: Gamma neurofeedback training in monkey's primary visual cortex

Authors: *L. A. CHAUVIERE[1], W. SINGER[2];
[1]Prof. Singer Lab., Max Planck Inst. For Brain Res., Frankfurt, Germany; [2]Singer Lab., Max Planck Inst. for Brain Res., Frankfurt am Main, Germany

Abstract: In humans, neurofeedback training has been successfully used to manipulate brain activity with EEG, fMRI and more recently with MEG recording techniques. In non-human primates, neurofeedback training has been successfully applied at the level of single cell (Schafer & Moore, 2011) and population activity to increase gamma-band activity in the primary motor cortex (Engelhard et al., 2013). In the current study, we chronically implanted two awake behaving macaque monkeys with 32 adjustable microelectrodes (Gray Matter Research) in the primary visual cortex and extracted the power of gamma oscillations within a narrow frequency band (± 15 Hz) from the local field potentials (LFPs) of one electrode. Monkeys that had been trained to fixate were rewarded if they maintained gamma band activity above a given threshold for at least a second while fixating. Correct performance was signaled by a tone and monkeys could increase their reward if able to prolong the tone. Both monkeys were able to increase the power and duration of gamma oscillations above the baseline level once feedback and reward were provided. Control experiments showed that it was also the case without auditory feedback, for trials/sessions when only reward was provided to the monkeys. Increasing the threshold for reward over subsequent trials/sessions led to a gradual increase of the feed back effects, suggesting that the animals learnt to control amplitude and maintenance of gamma oscillations in V1. It is to be expected that further analysis of the responses from the non-conditioned recording sites and the concomitantly registered unit activity will provide further insights into the mechanisms mediating the internally generated enhancement of gamma oscillations.

Disclosures: L.A. Chauviere: None. **W. Singer:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.02/CC72

Topic: F.02. Animal Cognition and Behavior

Support: Sakigake, JST

Title: Visual-cued fear conditioning of mice and the change in the neuronal firing property in the visual cortex

Authors: *M. K. YAMADA[1,2], T. EBINA[2], Y. WADA[3,4];
[1]Isotope Sci. Ctr., The Univ. of Tokyo, Tokyo, Japan; [2]RIKEN, BSI, Saitama, Japan; [3]Isotope Sci. Ctr., Tokyo, Japan; [4]The Res. Ctr. for Advanced Sci. and Technology,The Univ. of Tokyo, Tokyo, Japan

Abstract: Primary visual cortex is one of the most suitable areas of the brain for observation of neuronal firing properties by sensory input in the living animal. The cortical neuronal activity should be changed by visual stimulation, because visually evoked local field potential (VEP) in the visual cortex has been known to be altered in 24 hours by the repetitive stimulation using moving grating toward single-direction. We combined fear-conditioning with this type of visual stimulation, hoping that the footshocks in combination of the moving grating might alter the visual representation faster and more faithfully. Another good point of this method is that the attentiveness and normal sight in each individual mouse in addition to the associative learning at the conditioning period can be certified by change in the freezing behavior. The moving grating is the best known stimulation for the cell-firing in the primary visual cortex, thus we used that toward a single-orientation for the fear-conditioning of freely moving mice. As the result of giving footshocks in combination with moving grating, mice showed freezing behavior selectively to that visual stimulation. The freezing was observed at 3 hr after the conditioning and the level of the selectivity was increased from 3hr to 24hr. That is, after 24 hr, the difference of freezing time between to the moving grating and to the environment, so-called context, become significant. This result means that the mice can be conditioned to a visual stimulation such as the moving grating toward single-orientation in terms of fear response. We named this system as the visual-cued fear conditioning (vFC). Under the multi-photon microscope, mice with GCaMP3-expression in the excitatory neurons were given footshocks in combination with moving grating for a specific direction and the neuronal calcium levels were observed through the cranial window, before, during and after the procedure. (Mouse: NEX-Cre x Gt (ROSA)26Sortm1.1 (CAG-GCaMP3) Imayo; ref, Ebina et al. 2014, Cell Reports 9, 1896-1907). Change in the firing properties that might suggest, at least in part, network mechanism for cortical plasticity will be presented.

Disclosures: **M.K. Yamada:** None. **T. Ebina:** None. **Y. Wada:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.03/CC73

Topic: F.02. Animal Cognition and Behavior

Support: NEI Grant R00EY021503

NSF GRFP to Jaclyn Durkin

Title: Coherence of thalamocortical oscillations during sleep is required for consolidation of cortical plasticity in the visual system

Authors: *J. M. DURKIN[1], A. SURESH[2], J. COLBATH[1], S. J. ATON[1];
[1]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI; [2]Univ. of Chicago, Chicago, IL

Abstract: Orientation Specific Response Potentiation (OSRP) is a form of cortical plasticity in primary visual cortex (V1), which is initiated by waking visual experience and dependent on subsequent sleep. OSRP is accompanied by an increase in V1 neuron firing following visual stimulus presentation. We have previously found that optogenetic silencing of cortical feedback to the thalamus, which is hypothesized to be crucial for thalamocortical synchrony, during Non Rapid Eye Movement (NREM) sleep disrupts consolidation of experience-dependent cortical plasticity. To understand what mechanisms underlie this disruption, we performed dual site chronic recordings of individual visual thalamus (LGN) and V1 neurons under normal conditions and optogenetic silencing of cortical feedback following initiation of cortical plasticity. Preliminary data suggests that following initiation of cortical plasticity, LGN experiences increases in neuronal firing and optogenetically silencing cortical feedback during NREM sleep eliminates this increase in LGN firing. These preliminary findings suggest that synchrony between cortex and thalamus may be critical for maintaining the increase in LGN firing, which in turn may drive plasticity in visual cortex during NREM sleep.

Disclosures: J.M. Durkin: None. **A. Suresh:** None. **J. Colbath:** None. **S.J. Aton:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.04/CC74

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

Alberta Innovates Health Solutions

NSERC Discovery

Title: Hardware and software platform for spatial sequential learning in rodents

Authors: *D. R. EUSTON[1], H. W. STEENLAND[1,2];
[1]Univ. Lethbridge, Lethbridge, AB, Canada; [2]Neurotek innovative technology, Toronto, ON, Canada

Abstract: The testing equipment used in electrophysiology experiments is typically custom-built for a specific task and offers little flexibility. Given the time and effort involved in building such systems, it would be desirable to have control systems that could be quickly re-configured. Such systems need to be flexible enough to be altered for specific experiments and robust enough to control and receive information from the animal, experimenter and the data acquisition system. The field programmable gate array (FPGA) offers a great opportunity to control behavioral experimentation, conferring robust hardware control and sensor integration with the benefits of rapid configuration via software. The FPGA is a high-speed, customizable, parallel processing chip. Here we demonstrate the feasibility of using a National Instruments FPGA technology for a behavioral control system involving a spatial sequence task. A graphical user interface was developed in visual basic, so the experimenter could control the FPGA and receive camera-based tracking information from a data acquisition system. In addition, cable management hardware was developed for plug and play setup. This hardware connects the FPGA systems with lights, sounds, brain stimulation reward, timestamps, clock counts, and multiplexing audio control. Rats with implanted arrays of recording and stimulating electrodes could be taught to navigate a sequence task based on memory of light locations around a circular arena in a number of different spatial sequences or configurations.

Disclosures: D.R. Euston: None. **H.W. Steenland:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.05/CC75

Topic: F.02. Animal Cognition and Behavior

Support: LKS Biomedical Research Seed Fund

SRFDP-HKRGC joint grant (M-CUHK409/13)

HKRGC-GRF grant (14119214)

Title: Neuronal ensemble dynamics in layer 5b of primary motor cortex during motor learning

Authors: *Q. LI[1], H. KO[2,3], D. CHAN[1], G. ARBUTHNOTT[4], Y. KE[1], W. YUNG[1];
[2]Chow Yuk Ho Technol. Ctr. for Innovative Medicine, Fac. of Medicine, Fac. of Med., [3]Lui Che Woo Inst. of Innovative Med., [1]The Chinese Univ. of Hong Kong, Hong Kong, China; [4]Okinawa Inst. of Sci. and Technol. Grad. Univ., Okinawa, Japan

Abstract: The primary motor cortex (M1) comprises functionally heterogenous neuronal populations, with some encoding motor-related parameters such as direction and velocity. Recent evidence suggests that specific motor memories can be represented by sub-populations of neurons in layer 2/3 and layer 5a of M1. However, it is unknown how this upstream information is eventually conveyed to and reflected in the neuronal ensemble of the output layer 5b. Based on extracellular multi-unit recordings from conscious freely moving rats, we investigated the dynamics of layer 5b pyramidal neurons during learning of a motor skill, namely, the forelimb reaching for food task. We found a subpopulation of task-recruited layer 5b neurons that not only became more movement-encoding during motor learning, but their activities were also more temporally aligned to motor execution with a timescale of refinement in tens of milliseconds. These changes were accompanied by the emergence of a stable and task-specific structure of activity correlation, as well as more reproducible population neuronal activities with higher collective predictive power of motor output. Interestingly, local cortical depletion of dopamine by 6-hydroxydopamine injection degraded motor learning performance and disrupted the emergence of these reproducible spatiotemporal patterns of activities. Our results suggest that the recruitment of L5b neurons in a dopamine-dependent manner is crucial for the generation of more temporally aligned output signal from M1 to downstream circuitry, and may represent a mechanism underlying the enhanced precision of movement during motor learning.

Disclosures: **Q. Li:** None. **H. Ko:** None. **D. Chan:** None. **G. Arbuthnott:** None. **Y. Ke:** None. **W. Yung:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.06/CC76

Topic: F.02. Animal Cognition and Behavior

Support: W81XWH-08-1-0661

Title: Effects of single prolonged stress: a ptsd validated animal model, on pre- and post-synaptic marker expressions in fear-processing neurocircuitry

Authors: *E. RODRIGUEZ[1], I. LIBERZON[2];
[2]Psychiatry, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Post-traumatic stress disorder (PTSD) is a severely debilitating anxiety disorder. Core deficits of PTSD include extinction retention and contextual processing. Functionally, prefrontal cortex (PFC)-hippocampus (Hpc)- amygdalae circuitry is critical for these processes. Prolonged exposure to stress or stress hormone has been shown to lead to synaptic atrophy in lab animals. Patients with depression have also been shown to have significant decreases in synaptic

arborization, synapse numbers and expression of markers of synaptic function. To understand synaptogenesis' role in PTSD relevant fear processing of extinction recall we measured synaptogenesis markers following SPS exposure alone as well as in combination with fear learning resulting from fear conditioning, extinction and extinction recall. Synaptogenesis were measured in PTSD relevant neurocircuitry (mPFC, Hpc, and amygdala) using quantitative PCR. Within these regions, both pre- and post-synaptic signaling relevant molecules were measured: Syn1, PSD95, MAP2, GluR1 and BDNF. SPS alone significantly affected expression of BDNF and MAP2 within mPFC and the hippocampus. SPS and fear learning had a significant interaction in the expression of MAP2 and Syn1 in all three areas studied. Dendritic remodeling would represent a modifiable target that could lead to viable treatments as well as furthering our understanding of etiology of this disorder. Treatment targeting dendritic remodeling is currently being investigated for multiple disorders.

Disclosures: E. Rodriguez: None. **I. Liberzon:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.07/CC77

Topic: F.02. Animal Cognition and Behavior

Support: CIHR Grant

NSERC Grant

Title: Microcircuitry and physiology of the lateral pallium of a weakly electric fish

Authors: *A.-T. TRINH, E. HARVEY-GIRARD, L. MALER;
Univ. of Ottawa, Ottawa, ON, Canada

Abstract: In the weakly electric gymnotiform fish, Apteronotus leptorhynchus, the dorsolateral pallium (DL) is implicated in learning and memory and considered to be homologous to medial and/or dorsal pallium (hippocampus/cortex). Previous studies reveal that there is an expansive representation of sensory input in DL similar to that seen in thalamo-cortical projections. The gymnotiform DL has an apparently uniform architecture composed of randomly distributed multipolar neurons. We used micro neurotracer injections in order to study the microcircuitry of DL. Surprisingly, we have shown that the intrinsic connectivity of DL is highly organized into horizontal bands and vertical columns of excitatory synaptic connections. The horizontal connections subdivide DL into narrow (60 μm) overlapping cryptic layers that are symmetric, sparse and random; the probability of connectivity between neurons drops exponentially with distance. Using a directed random graph model, we have found that the minimal distance for strong connectivity in the horizontal plane is 100 μm. The vertical connectivity on the other

hand, is highly asymmetric with superficial DL cells preferentially projecting towards deeper cells suggesting a cryptic columnar organization. Our experimental data suggest that the overlapping cryptic columns have a width of 100 μm, which is in agreement with the minimal distance required for strong connectivity. The connectivity of DL and the expansive representation of its input, combined with the strong expression of NMDA-Rs by its cells, are in agreement with theoretical ideas regarding the cortical computations of pattern separation and memory storage via recurrent networks. To further investigate if DL can support recurrent networks, we switched to whole-cell recording in acute brain slices in order to characterize the neurons in DL. Preliminary results suggest that DL cells are typically inactive due to having a low resting membrane potential and a high spike threshold. Initial characterization has also revealed that these neurons have a persistent inward Na+ current, strong Ca2+ currents and that spikes are followed by a strong after-hyperpolarizing potential (AHP). Using a pharmacological approach, we have found that the AHP is caused by the SK channels known to be abundantly expressed in DL neurons. The AHP leads to prominent spike-frequency adaptation (SFA) of the response to step depolarization of DL neurons. The combination of a strongly connected network with slow NMDA-R mediated excitation and negative feedback in the form of SFA suggests that DL meets the theoretical requirements for memory storage via bump attractor networks.

Disclosures: **A. Trinh:** None. **E. Harvey-Girard:** None. **L. Maler:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.08/CC78

Topic: F.02. Animal Cognition and Behavior

Support: Global COE Program

FIRST program

Brain/MINDS

Title: Performance of common marmosets in a delayed positional matching-to-sample task

Authors: ***Y. YAMAZAKI**[1,3], M. SAIKI[3], M. INADA[3], S. WATANABE[2], A. IRIKI[3];
[1]Keio Advanced Res. Centers, [2]Dept. of Psychology, Keio Univ., Tokyo, Japan; [3]Lab. for Symbolic Cognitive Develop., RIKEN BSI, Saitama, Japan

Abstract: Working memory is used to solve various daily cognitive problems by maintaining information for some time and then by refreshing this information after certain purposes are achieved. In the present study, we explored common marmosets' ability to perform a delayed positional matching-to-sample (DPMTS) task in a controlled environment using operant

respondings to the touch-sensitive screens. The DPMTS task requires the subjects to respond to the sample stimulus and to select one of two comparison stimuli with a position matching that of the sample stimulus after a programmed delay period. Positional arrangement of the sample and comparison stimuli, which were quasi-randomly determined in each trial, was employed to prevent the subjects from using any strategies based on their own body positions or orientations. The delay intervals between presentations of the sample and comparison stimuli were fixed at 0.5 and 1 sec in the initial phases and were then varied between 5 intervals per delay set (e.g., 0.5, 1, 2, 4, and 8 sec) intermixed in a session. The longest delay interval within a set was gradually increased after the marmosets achieved the criterion of each task. The subjects were successfully trained in the procedure and showed accurate performance, and 4 out of 6 subjects showed above criterion performance even following delays of more than 100 sec. Difference of sex and experimental history had little effect on the delayed performance. The performance was declined as the longest delay intervals prolonged. The response times in the trials suggested that they used different strategies depending on the delay interval length. With the shorter delay intervals, they tended to wait for the comparison stimuli to be presented in front of the response panel. Accuracy was well preserved even when positional memory was disturbed during the long delay intervals. Thus, the present study shows the robust ability of common marmosets in a task requiring positional memory, which is related to their foraging strategy observed in the wild.

Disclosures: Y. Yamazaki: None. **M. Saiki:** None. **M. Inada:** None. **S. Watanabe:** None. **A. Iriki:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.09/DD1

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS063494

Title: Associative memory storage and synaptic connectivity in homeostatically constrained networks of excitatory and inhibitory neurons

Authors: *A. B. STEPANYANTS[1], J. CHAPETON[2], R. GALA[1];
[1]Physics, Northeastern Univ., Boston, MA; [2]NIH, Bethesda, MD

Abstract: Learning and memory formation in the brain are accompanied by changes in synaptic connectivity of the underlying neural network. Yet, the specific effects of learning on connectivity are not understood. There are some connectivity features that are ubiquitously present in local cortical networks. These features include very sparse connectivity of excitatory neuron axons, much denser connectivity established by the axons of many inhibitory neuron

classes, and stereotypically distributed connection weights. To understand if such features can form as a consequence of learning we considered a biologically constrained, exactly solvable model of associative memory storage. The model is based on the hypothesis that cortical areas in the adult function in a steady-state where learning is accompanied by forgetting. The model can include homeostatic constraints on the number or weight of functional synaptic connections and can accommodate multiple excitatory and inhibitory neuron classes. We find that in spite of multiple neuron classes, functional connections between potentially connected cells are realized with less than 50% probability if the presynaptic cell is excitatory and generally a much greater probability if it is inhibitory. We also find that constraining the overall weight of presynaptic connections leads to Gaussian connection weight distributions that are truncated at zero. In contrast, constraining the total number of functional presynaptic connections leads to non-Gaussian distributions, in which weak connections are absent. These theoretical results are compared with experimental measurements of connection probabilities and weights from various cortical areas and species, suggesting that stereotypic features of adult connectivity can form despite functional differences among brain areas and diverse learning experiences of individuals.

Disclosures: **A.B. Stepanyants:** None. **J. Chapeton:** None. **R. Gala:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.10/DD2

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant P01NS07497201

HFSP fellowship LT000446/2013-L

Title: Somatostatin interneurons exhibit diverse activities during motor learning

Authors: ***A. ADLER**, W.-B. GAN;
Skirball Inst. of Biomolecular Med., New York Univ., New York, NY

Abstract: Somatostatin (SST) cells account for approximately 30% of cortical GABAergic inhibitory interneurons. Their cell bodies are located in layers 2 to 5 and their axons make extensive contacts with the apical tuft dendrites of pyramidal cells. SST neurons have been shown to directly block the initiation of dendritic calcium spikes in response to sensory stimuli. Furthermore, active or passive whisker stimulation leads to hyperpolarization and reduced activity of all SST cells. These lines of evidence suggest that during sensory processing, the activities of SST cells are inhibited to allow for enhanced excitability and sensory processing. Here we explored the function of SST cells in the motor cortex during motor learning by examining their responses to different motor learning tasks. *In vivo* calcium imaging was used to

monitor the activity of SST cells in mice subjected to running forward or backward on a treadmill. SST-IRES-Cre mice were injected with adenoviruses expressing the genetically encoded calcium indicator, GCaMP6 specifically in layer 2/3 of the forelimb region of the primary motor cortex. We found that SST interneurons displayed diverse response patterns, with both increased and decreased activities in response to forward and backward running. The responses to forward and backward running within individual SST cells were highly selective as different motor learning tasks induced significantly different activity patterns. This response selectivity was manifested in the cells' response polarity, i.e. if a SST cell increased its activity to forward running, it was likely to decrease its activity to backward running and vice versa. Our results suggest that unlike sensory cortex, SST neurons in the motor cortex do not function as a single dimension uniform unit during motor learning.

Disclosures: A. Adler: None. **W. Gan:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.11/DD3

Topic: F.02. Animal Cognition and Behavior

Support: European Research Council, Advanced Grant

Swiss National Science Foundation

Title: Membrane potential dynamics of specific cortico-cortical projection neurons correlated with goal-directed behaviour

Authors: *T. YAMASHITA[1,2], C. C. H. PETERSEN[2];
[1]Res. Inst. of Envrn. Med., Nagoya Univ., Nagoya, Japan; [2]Brain Mind Inst., EPFL, Lausanne, Switzerland

Abstract: How sensory information is segregated in primary sensory cortex to generate multiple processing streams towards other cortical areas is a fundamental issue in studying sensory processing. However, the cellular and synaptic mechanisms underlying such distinct signalling pathways are poorly understood. By making *in vivo* whole-cell recordings in primary somatosensory barrel cortex (S1) of awake behaving mice, we have previously shown that S1 neurons projecting to primary motor cortex (M1) and those projecting to secondary somatosensory cortex (S2) have different intrinsic membrane properties and exhibit markedly different membrane potential dynamics during behaviour (Yamashita et al., Neuron, 2013). Here we questioned how output signals from S1 to M1 or S2 would be changed by learning a simple detection task where evoked S1 activation is needed for execution of goal-directed, licking behaviour (Sachidhanandam et al., Nature Neuroscience, 2013). Early sensory-evoked responses

in the M1- or S2-projecting neurons were not significantly changed by learning the task. In hit trials, late depolarization at 100-300 ms after whisker deflection, however, became larger in S2-projecting neurons after learning, but not in M1-projecting neurons. In well-trained mice, S2-projecting neurons, but not M1-projecting neurons, showed depolarization during spontaneous non-rewarded licking which was marginal during licking in naive water-deprived animals. Interestingly, these learning-induced changes in membrane potential dynamics in S2-projecting neurons were observed within a few hours at the first training day, in a subset of mice which quickly learned the task. These results suggest that learning the S1-related detection task reorganizes S1 microcircuits to specifically enhance "ventral stream" S1-S2 signals correlated to goal-directed motor outputs.

Disclosures: T. Yamashita: None. **C.C.H. Petersen:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.12/DD4

Topic: F.02. Animal Cognition and Behavior

Support: DBTO/BCN/BJ/0402

DSTO/BCN/BJ/1102

DSTO/BCN/BJ/1297

JTT/MUM/INST/IIOS/2013-14/0033

CSIR- 09/079(2590)/2012-EMR-l

Title: Sensitive method to follow dynamics of remote memory in mice using social transfer of food preference

Authors: *A. SINGH, S. KUMAR, V. SINGH, S. SHRIDHAR, J. BALAJI;
Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: Long term memories have been hypothesized to undergo various transitions in its content as well as the loci of storage as memory gets consolidated. Recent studies have shown that synaptic activation and gene expression in neocortex is required even during recent memory formation for the stable formation of remote memory. This is in contrast with the conventional notion that all the plasticity mediated events happen in hippocampus during formation of recent declarative memories. In this context, we hypothesize that neocortical synaptic activation at recent time point precedes transcriptional activation of neurons and stabilization of remote memory trace. Using behavior paradigm of social transfer of food preference (STFP) we have

observed that memory of demonstrated flavor can be retrieved in mice as long as forty five days after behavior. This provides us with a window of sufficient time to observe the dynamics of consolidation events happening during and post STFP training. We incorporate novel photonic tools and fluorescent probes to follow and test our hypothesis.

Disclosures: **A. Singh:** None. **S. Kumar:** None. **V. Singh:** None. **S. Shridhar:** None. **J. Balaji:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.13/DD5

Topic: F.02. Animal Cognition and Behavior

Support: Future Systems Healthcare Project of KAIST

Title: Excitability-dependent memory allocation and manipulation in feedforward neural network model

Authors: ***P. KANCHANAKANOK**, W. CHOI, S.-B. PAIK;
Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Memory plays an important role in human life and relates to learning ability. It has been reported that memory allocation is influenced by intrinsic neural properties, such as neural excitability, and can be manipulated by varying those parameters (1, 2). Particularly, neural excitability is known to be a critical factor in the allocation of fear memory in the amygdala (3). To understand the relationship between neural excitability level and selective memory allocation, we developed a simple neural network model and observed changes in memory traces during excitability manipulation. Our hypothesis is that higher neural excitability would result in higher chance to be involved in memory allocation with strengthened feedforward synaptic connections between input and target neurons. Moreover, we assume that recurrent connections would play a critical role in the transition of memory allocation in the circuit so that a feedforward-only network model cannot reproduce the transition observed in animal studies. To confirm the idea, we developed a feedforward-only network model of leaky integrate-and-fire neurons which receives spike inputs from sparse-and-random synaptic connections. We first confirmed that our network model could learn and memorize a specific input pattern with a simple spike timing dependent plasticity rule. Next, to test the effect of high neural excitability, we intentionally increased the resting membrane potential of a group of neurons (group A) and observed that group A neurons have noticeably higher probability to be involved in the memory trace. After this, to simulate the memory transition between neuron groups, we increased the resting potential of another neuron group (group B) while group A's resting potential was turned back to a normal

state. After memory training simulation, we found that group B's response could not surpass group A's response for memorized pattern and that both group A and B cells were involved in the memory trace. Our result suggests that the memory allocation can be achieved by simple Hebbian learning rule, and the achieved memory trace highly depends on neural excitability level. It also shows that memory allocation in a neural network is variable so that the involved neural population for particular memory may not be unique. Moreover, in the absence of recurrent connections, memory transition could not be readily achieved. Further studies with a recurrent network model would show a mechanism of competitive activities among in-layer cells in memory allocation and transition. Reference 1.Redondo, Nature, 513, 426-430 (2014) 2.Yiu, Neuron, 83, 722-735 (2014) 3.Zhou, Nat. Neurosci., 12, 1438-1443 (2009)

Disclosures: P. Kanchanakanok: None. **W. Choi:** None. **S. Paik:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.14/DD6

Topic: F.02. Animal Cognition and Behavior

Support: EC-FP7-604102

Title: Neural computation with assemblies and assembly sequences

Authors: *C. POKORNY, R. LEGENSTEIN, W. MAASS;
Inst. for Theoretical Computer Sci., Graz Univ. of Technol., Graz, Austria

Abstract: Simultaneous recordings from large numbers of neurons have found in many cortical areas of mice stereotypical spatio-temporal patterns in their firing activity, commonly referred to as assemblies, assembly sequences, or trajectories of network states. These characteristic spatio-temporal patterns (abbreviated STPs) of neural activity occur both spontaneously and stimulus evoked in primary sensory areas A1, V1, S1 (Luczak et al, 2007; Sadovsky et al., 2014; Miller et al., 2014). They have also been found in areas PFC (Fujisawa et al., 2008) and PPC (Harvey et al., 2012) as neural traces of specific behaviours and movement plans. We address in this poster the question what these experimental data tell us about the organization of brain computations, and how models for brain computations should be modified in order to take them into account. This question is of particular interest, since the recorded activity is quite different from an asynchronous irregular firing regime (that was previously proposed in theoretical studies as best supporting neural computation). The experimental data also suggests that computations on the network level cannot be described adequately by attractor networks, since one finds stereotypical TRANSIENT activity patterns, rather than convergence to specific network states or limit cycles. We report in this paper first results of a new computational theory that is based on the

assumption that the experimentally found stereotypical STPs play an important role in network computations. We show that STPs enable computation and learning in a context-dependent manner. We also show that STPs may enable selection and switching between different functional networks for specific computational tasks. Our computational theory builds on concepts and mathematical tools provided by recent work on computations in cortical microcircuit models with biologically realistic amounts of noise (S. Habenschuss et al., 2013, Klampfl , 2103). REFERENCES S. Fujisawa, A. Amarasingham, M.T. Harrison, G. Buzsáki; Nat. Neuroscience, 2008 S. Habenschuss, Z. Jonke, W. Maass; PLOS Comp. Biol., 2013 C.D. Harvey, P. Coen, D.W. Tank; Nature, 2012 S. Klampfl and W. Maass; J. of Neuroscience, 2013 A. Luczak, P. Barthó, S.L. Marguet, G. Buzsáki, K.D. Harris; PNAS, 2007 J.E.K. Miller, I. Ayzenshtat, L. Carrillo-Reid, R. Yuste; PNAS, 2014 A. Sadovsky and J.N. MacLean; J. of Neuroscience, 2014

Disclosures: C. Pokorny: None. **R. Legenstein:** None. **W. Maass:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.15/DD7

Topic: F.02. Animal Cognition and Behavior

Support: DBTO/BCN/BJ/0402

DSTO/BCN/BJ/1102

DSTO/BCN/BJ/1297

JTT/MUM/INST/IIOS/2013-14/0033

Title: Differential influence of neocortical networks (mental schema) in relational and abrupt learning

Authors: *V. SINGH[1], S. SHRIDHAR[1], S. KUNDU[1], R. BHATT[1], S. SAM[2], A. SINGH[1], S. KUMAR[1], J. BALAJI[1];
[1]Ctr. for Neurosci., [2]Ctr. for Nano Sci. and Engin., Indian Inst. of Sci., Bangalore, India

Abstract: Memories of related events and facts are stored in the form of neocortical associative networks (mental Schema) and they help in rapid consolidation of new memories that are similar in character. Although neocortex is shown to be involved in rapid learning of small modifications to existing schema, it is not clear if this is true for learning larger information sets with content richness similar to the initial schema. We show that a pre-existing schema may or may not influence such new learning depending on the mode of training. Further, we show that if the novelty is introduced in relation to pre-formed schema (relational learning) the learning is

rapid even when acquiring/forming a new memory of similar complexity. We established event arena based behavioural paradigm in mice to test the schema formation and used it to teach mice two maps of equal complexity. Initially, one map was taught while the other was taught later in relation to the first. The animals demonstrated rapid encoding of the second map (presented later).We also found that relational learning is faster than incremental learning. We will also present our results for the role of such neocortical networks in advanced cognitive scenarios such as problem solving.

Disclosures: **V. Singh:** None. **S. Shridhar:** None. **S. Kundu:** None. **R. Bhatt:** None. **S. Sam:** None. **A. Singh:** None. **S. Kumar:** None. **J. Balaji:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.16/DD8

Topic: F.02. Animal Cognition and Behavior

Support: NIH-MH081153

Title: Tracking changes in spikes rates as a function of t ask covariates within a single session

Authors: *F. A. MUNOZ[1], G. JENSEN[2,1], B. KENNEDY[3], V. P. FERRERA[1,4], H. TERRACE[2];
[1]Neurosci., [2]Psychology, [3]Neurosurg., [4]Psychiatry, Columbia Univ., New York, NY

Abstract: The development of analytical tools for correlating neu rophysiological recordings with behavior during learning has been hampered by pr actical and conceptual roadblocks. Although current analytic methods permit the e xperimental demonstration of tuning functions that have previously be en learned over the course of a lengthy training regimen, the processes under lying learning itself remain mysterious. Of particular note is the problem of parameter estimation in non-stationary data: Although kernel smoothing procedure s have enjoyed great success in characterizing firing over the course of trials du ring stable performance, they are not appropriate for evaluating f iring rates during the initial acquisition of a behavior, during which neuronal acti vity is continuously changing. We propose a novel method for mapping firings rates ove r the course of a single experimental session, using multivariate adaptive regre ssion splines. This nonparametric approach not only permits inference about changing firing rates within individual trials, but also tracks the evolution of firing rates over the course of a single session. This permits trials to be understoo d not as independent cases, but instead as a continuum that unfolds over the dur ation of a session. This procedure also bypasses longstanding difficulties surr ounding bandwidth selection. We use this procedure to investigate real-time changes in firing rate, revealing how single neurons change their behavior thro ughout

initial training, rather than as a result of training that was performe d at some prior time. This lets us identify the precise moments in a session at which tuni ng functions emerge, differentiating baseline firing from the tuned firi ng that reflects learning. We demonstrate this procedure on recordings from cells in p arietal area LIP in monkeys during a transitive inference task. In each session, subjects were presented with never-before-seen photographic stimuli, and learned how those stimuli were ordered using trial and error. This per mits modeling of response accuracy over the course of a session, based on the firing r ate. Insofar as neurons become tuned to task features (such as stimulus pos ition and order), we are able to show the timing and magnitude of changes i n tuning within the session. When used in concert with temporally consistent tr ial structures, this procedure enables a host of hitherto uninvestigated quest ions about learning processes to be investigated.

Disclosures: F.A. Munoz: None. **G. Jensen:** None. **B. Kennedy:** None. **V.P. Ferrera:** None. **H. Terrace:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.17/DD9

Topic: F.02. Animal Cognition and Behavior

Title: Comparison of classical and operant conditioning using behavior and electrophysiology during auditory learning tasks

Authors: *M. GOLDSCHMIDT[1,2], A. KOLODZIEJ[1,2], A. SCHULZ[1], F. OHL[1,2,3];
[1]Leibniz Inst. For Neurobio., Magdeburg, Germany; [2]Otto von Guericke Univ., Magdeburg, Germany; [3]Ctr. for Behavioral Brain Sci., Magdeburg, Germany

Abstract: To obtain a more fundamental understanding of the role and the mechanism of cortico-striatal interaction underlying learning, two different forms of learning will be compared: A classical conditioning paradigm (fear conditioning) and an instrumental conditioning paradigm (avoidance learning). The planned approach provides a scenario to investigate the phenomenon of Pavlovian-instrumental transfer (PIT), a process where in the first phase an association is made between a conditioned stimulus (CS) and an unconditioned stimulus (US) by classical conditioning and in the second phase there is a transfer from classical to instrumental. Therefore, we designed a combined learning experiment with male C57BL/6J and NMRI mice. In this experiment one group of animals was trained initially in a modified fear conditioning paradigm and subsequently in a customized two-way active avoidance paradigm, and a second group in a reversed order. To discover possible similarities and differences between these two basic learning scenarios we will use behavioral analyses like scoring of freezing during fear conditioning, recording of hit-rate and false-alarm-rate during avoidance conditioning. In

addition to the behavioral experiments, simultaneous electrophysiological recordings of local field potentials from auditory cortex and ventral striatum will be obtained from the awake behaving animals. This approach allows dissociating the specific functions of these two behavioral brain structures and their interplay in classical and instrumental conditioning.

Disclosures: **M. Goldschmidt:** None. **A. Kolodziej:** None. **A. Schulz:** None. **F. Ohl:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.18/DD10

Topic: F.02. Animal Cognition and Behavior

Support: DFG SPP 1665

Title: Cortical learning and moudulation of mesoscopic cortical activity patterns in the Mongolian gerbil

Authors: ***K. TAKAGAKI**[1], G. ARIAS-GIL[2], M. T. LIPPERT[2], F. W. OHL[2];
[1]Leibniz Inst. for Neurobio., Magdeburg, Germany; [2]Leibniz Inst. for Neurobiology, Magdeburg, Magdeburg, Germany

Abstract: Transcranial DC (tDC) stimulation has been known to modulate human sensation since the early 1960s. Recently, there is a resurgence of this method, and many recent studies have documented that tDC modulates human learning. Besides the basic neuroscience applications in learning and memory, tDC is also considered to be a promising treatment modality for everything from stroke rehabilitation (modulation of plasticity) to depression (modulation of mood), due to both its ease of application and its global modulatory effects. However, very little is known about the effects of tDC on the neocortex, and how these neocortical effects lead to changes in learning and memory. We investigate the mechanisms of these effects on learning and memory, both on the behavioral scale, using a cortically-dependant auditory discrimination task in Mongolian gerbils, and on the mesoscopic physiology scale using electrocorticography and voltage-sensitive dye imaging.

Disclosures: **K. Takagaki:** None. **G. Arias-Gil:** None. **M.T. Lippert:** None. **F.W. Ohl:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.19/DD11

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS069679

Rita Allen Foundation

Klingenstein Fund

Title: A head-fixed behavioral paradigm for studying whisker-mediated object recognition in mice

Authors: *C. RODGERS, A. KHANNA, P. CALAFATI, R. M. BRUNO;
Columbia Univ. Med. Ctr., New York, NY

Abstract: Rodents rely on whisker-mediated touch to explore their environment. By sweeping their whiskers across objects, similar to the way in which we palpate objects with our fingertips, rodents can identify object location, texture, and shape. The "novel object recognition" task, widely used in psychiatric research, challenges freely moving rodents to identify unfamiliar objects; however, it is typically unclear what sensory modality and strategy they use to do so. In contrast, head-fixed behavioral paradigms for studying rodent somatosensation are generally used to study object location and texture, not shape. Therefore, we have developed a head-fixed behavioral paradigm for studying object recognition that is amenable to modern imaging and recording techniques. Preliminary results indicate that mice are able to identify objects differing along a single tactile dimension in their whisker field. We ensure that mice do not usual visual cues by training them in total darkness. Trimming off all the whiskers reduced performance to chance, demonstrating that mice indeed use their whiskers to perform this task. This paradigm is useful for examining the circuit activity underlying object recognition and how it relates to processing in other sensory systems.

Disclosures: C. Rodgers: None. **A. Khanna:** None. **P. Calafati:** None. **R.M. Bruno:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.20/DD12

Topic: F.02. Animal Cognition and Behavior

Support: National Basic Research Program 2013CB531304

National Basic Research Program 2011CB504405

Natural Science Foundation China 30990261

Natural Science Foundation China 81171033

Natural Science Foundation China 81471123

Title: Neuron-specific mechanisms for unilateral learning and bilateral memory recalls

Authors: *J. H. WANG[1], R. FAN[2], L. CHEN[2], Z. GAO[1];
[1]The Inst. Biophysics, Beijing, China; [2]Bengbu Med. Col., Bengbu, China

Abstract: After sensory signals and operative skills are learnt by unilateral limb, they are retrieved in bilateral limbs, such as the hand-writing can be performed by both hands after learnt by a hand. This unilateral learning toward bilateral memory is critical for both-side limbs to be coordinate in response to the environmental changes. In terms of cellular mechanism for this unilateral learning to bilateral memory, the plasticity of the corpus callosum that connects two cerebral hemispheres is presumably involved. We aim to examine how the corpus callosum plasticity initiates the recruitment and refinement of different cortical neurons to encode the unilateral learning toward bilateral memory. To study cell-specific mechanisms, the mouse glutamatergic neurons were genetically labeled by yellow fluorescent protein and GABAergic neurons were green fluorescent protein. AAV-SynaptoTag-Cherry (a gift from Tom Shudoff) was injected into the barrel cortex corresponding to the training side of whiskers to trace its distribution in the contralateral side and the synapse formation under confocal cell imaging. Electrophysiological recordings in two sides of the barrel cortices *in vivo* were used to analyze how their neurons encode associative signals. Whole-cell recordings in the brain slices were used to assess plasticity in neuronal spiking and synaptic transmission. Pairing stimuli to mouse unilateral whiskers and olfaction led to odorant-induced motion in the bilateral whiskers and their motion frequencies were higher in the training side than non-training side. In the mice of expressing this bilateral signal retrieval, the axon innervation between both sides of the barrel cortices was strengthened and new synapses between them were formed. The neurons in both sides of the barrel cortices became able to process the newly learnt odor signal besides innate whisker signal. Their encoding patterns in response to two associative signals were different. The barrel cortical excitatory neuronal units were functionally increased, and the inhibitory neuronal units were decreased. The fact that the bilateral memory recalls are induced by unilateral learning indicates that the learning by the unilateral side leads to memory formation in the bilateral cortices. The new synapse formation and neuron-specific recruitment constitute the bases for both sides of the cortical neurons to process the odor and whisker signals for their associative storage and distinguishable retrieval in unilateral learning toward bilateral recalls.

Disclosures: **J.H. Wang:** None. **R. Fan:** None. **L. Chen:** None. **Z. Gao:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.21/DD13

Topic: F.02. Animal Cognition and Behavior

Support: F31 AA023141

P50 AA010761

Title: Regulation of goal-directed behavior by nucleus accumbens glutamate signaling

Authors: *J. M. BARKER[1], J. CHANDLER[2];
[1]Psychiatry, [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: The ability to flexibly regulate behavior is impaired in a number of neuropsychiatric disorders, including addiction. This loss of behavioral flexibility may involve a transition from goal-directed actions, where rewards are sought for their reinforcing properties, to habitual behaviors which are not performed in relationship to their outcome. Response strategy selection is mediated in part by corticostriatal circuitry. Previous research has implicated the infralimbic PFC (IL) in the expression of habitual reward seeking; loss of IL function restores goal-directed action in rodents that have acquired habitual reward seeking. Extinction learning has implicated IL projections to the nucleus accumbens shell (NAcS) in the regulation of reward seeking, but the downstream target of IL projections that mediate habitual behavior have not yet been assessed. Our recent research has implicated glutamate signaling in the NAcS in the expression of habitual behavior. In particular, we observed that mGluR2/3 agonism in the NAcS restores goal-directed reward seeking after the development of habitual behavior. To investigate the role of IL projections to the NAcS, we are utilizing a pharmacogenetic strategy. In particular, a virus expressing the inhibitory DREADD under control of the CAMKII promoter, which restricts expression to glutamatergic neurons, was injected into the IL. Findings indicate that inhibition of IL glutamatergic projections by agonism of the inhibitory DREADD prevent the expression of habitual behavior. This pharmacogenetic strategy is being employed to test the hypothesis that IL projections selectively to the NAcS are critical for habitual reward seeking.

Disclosures: J.M. Barker: None. **J. Chandler:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.22/DD14

Topic: F.02. Animal Cognition and Behavior

Support: NINDS Grant NS038890

Title: Ontogenetic changes in anterior cingulate cortical activity during trace eyeblink conditioning

Authors: *M. E. ELKIN, J. H. FREEMAN;
Univ. of Iowa, Iowa City, IA

Abstract: The ontogeny of trace eyeblink conditioning is believed to be dependent upon maturation of forebrain structures such as the anterior cingulate cortex (ACC) and hippocampus. (Goldsberry et al., 2015, J. Neurosci., 35:4238). Although neuronal responsiveness in the ACC during trace eyeblink conditioning has been studied in adults, there is a paucity of research concerning its role in the developing animals. The goal of the current study was to examine the development of neuronal activity in the ACC while developing rats were trained in trace eyeblink conditioning. We implanted moveable tetrodes in rat pups on postnatal days (P) 17, 19, 22 and 29. The rat pups were given one of day rest and then trained on trace eyeblink conditioning twice a day for three days, for a total of six sessions. Trace eyeblink conditioning involved presentation of a 250 ms tone conditioned stimulus CS, followed by a 500 ms stimulus-free trace interval which terminated in a 25 ms periorbital shock unconditioned stimulus (US). The rate of acquisition of trace conditioning increased across age groups. The spontaneous firing rate of ACC neurons also increased as a function of age. During conditioning trials there was a substantial developmental increase in the percentage of ACC neurons responsive to individual trial events (i.e., the CS, trace interval, US) and combinations of trial events from the first paired training session. The results indicate that the ACC undergoes developmental changes in neuronal coding of trial events.

Disclosures: M.E. Elkin: None. **J.H. Freeman:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.23/DD15

Topic: F.02. Animal Cognition and Behavior

Support: MH080005

Title: Anterior cingulate cortical control in visual attention

Authors: *J. KIM, E. A. WASSERMAN, L. CASTRO, J. H. FREEMAN;
Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: The dorsal anterior cingulate cortex (dACC) has been proposed to control selective attention. However, the mechanisms of how dACC neuronal firing and theta rhythms contribute to visual attention have not been examined. In the current study, dACC neuronal activity was

recorded from multiple tetrodes while the rat performed a dACC-dependent visual attentional task using a touchscreen apparatus. On every trial, the rats were required to attend to the task-relevant visual stimulus while ignoring a task-irrelevant stimulus. Neuronal spike activity and theta were analyzed during task event periods (i.e., pre-trial, cue-onset, cuing/maintenance, and choice button selection). Both spike activity and theta power decreased during correct trials. Specifically, the spike activity was inhibited during the initial cuing phase for task-relevant stimulus selection and theta power was suppressed in the middle of cuing for maintaining the task information. Cross-correlation analysis showed that spike firing preceded theta in the pre-trial and maintenance periods when the rat made correct choices, whereas theta preceded spikes on the incorrect trials. Also, coherence in the correct trials was higher than incorrect trials during the maintenance period. Lastly, there was higher phase synchrony between dACC spikes and theta during the pre-trial period of the correct trials. The results show that dACC activity should be orchestrated in time and phase before cue-onset for the successful performance. The synchronized dACC activity leads the suppression of both dACC and theta during the correct selection and maintenance of task information. The results suggest that inhibited, yet synchronous, dACC firing is necessary for rodent visual attention tasks.

Disclosures: **J. Kim:** None. **E.A. Wasserman:** None. **L. Castro:** None. **J.H. Freeman:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.24/DD16

Topic: F.02. Animal Cognition and Behavior

Support: NINDS Grant NS038890

Title: Ontogeny of hippocampal CA1 activity during somatosensory trace eyeblink conditioning

Authors: *M. E. GOLDSBERRY, J. KIM, J. H. FREEMAN;
Univ. Iowa, Iowa City, IA

Abstract: Trace eyeblink conditioning (EBC) involves the association of a conditioned stimulus (CS) with an unconditioned stimulus (US) over a stimulus-free trace interval. The inclusion of this trace interval necessitates the involvement of late-developing forebrain structures such as the hippocampus and medial prefrontal cortex. Until recently, it was believed that development of these structures was the rate-limiting factor in the developmental emergence of trace EBC. Indeed, hippocampal neuronal activity shows an age-related increase in both complexity and task responsiveness during trace EBC. However, recent work from our laboratory suggests that sensory system development may also play a role. Training with the earlier-developing somatosensory system results in an earlier emergence of trace EBC in rats. Whereas learning

with a tone CS does not develop until postnatal day (P) 24, vibration-trained pups show robust levels of learning as early as P21. This suggests that the development of sensory input to the hippocampus may influence the development of trace EBC. The goal of the current study was to examine the activity of CA1 pyramidal cells during the acquisition of trace EBC with an early-developing CS modality. Rat pups were trained on P17-19, P21-23, and P24-26 while neuronal activity was recorded with a custom-built four-tetrode drive. Training consisted of two sessions per day, for a total of six sessions of somatosensory trace EBC. As seen in auditory trace conditioning, CA1 neurons responded to a number of trial events. However, the proportion of responsive neurons was significantly greater in pups trained with a vibration CS. The source of this difference appeared to occur in the proportion of neurons that were responsive during the trace interval. Pups trained with a vibration CS had a greater number of CA1 cells that showed increases in activity during the stimulus-free trace interval. Moreover, the magnitude of neuronal responding was greater in pups trained with a vibration CS. Peaks in magnitude were evident both at CS onset and at the beginning of the trace interval. These findings suggest that sensory input to the hippocampus may be playing a role in the development of trace EBC.

Disclosures: M.E. Goldsberry: None. **J. Kim:** None. **J.H. Freeman:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.25/DD17

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NINDS NS038890 awarded to JHF

Title: Amygdala and prelimbic neuronal activity during retention of fear conditioning in developing rats

Authors: *K. L. BROWN, J. H. FREEMAN;
The Univ. of Iowa, Iowa City, IA

Abstract: Pavlovian fear conditioning has been used extensively to identify developmental changes underlying learning and memory. *In vivo* tetrode recording of neuronal activity has yielded important insights into developmental changes underlying associative motor learning [Ng and Freeman (2012), The Journal of Neuroscience, 32, pp. 6841-6850; Goldsberry, Kim, and Freeman (2015), The Journal of Neuroscience, 35, pp. 4238-4247], though *in vivo* recordings have yet to be applied to fear conditioning in developing rats. The present study is the first to use *in vivo* tetrode recordings during fear conditioning in developing rats. Postnatal day (P) 17 or 24 rats received presentations of white noise conditional stimuli (CS) and floor shock unconditional stimuli (US) in context A. Learned fear to the CS was measured by freezing to presentations of

the CS-alone in context B. Short-term retention was robust and comparable in rats at both age groups that had received paired (but not unpaired) CS-US presentations at training. However, long-term retention was robust only for P24 rats. Preliminary findings indicate robust, short-latency CS-related neuronal activity in the amygdala at testing in rats at both ages that had received paired CS-US training. In contrast, longer-latency CS-related prelimbic neuronal activity was evident only in rats that had received paired training on P24. These findings provide the basis for novel investigations into the mechanisms underlying developmental changes in memory retention and retrieval.

Disclosures: K.L. Brown: None. **J.H. Freeman:** None.

Poster

357. Learning and Memory: Cortical Circuits

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 357.26/DD18

Topic: D.14. Cerebellum: Central Physiology

Support: NINDS grant NS088567

Title: Amygdala modulation of cerebellar learning

Authors: *S. J. FARLEY, J. H. FREEMAN;
Dept of Psychological and Brain Sci., The Univ. of Iowa, Iowa City, IA

Abstract: We examined the mechanisms underlying amygdala modulation of cerebellar learning. Rats were trained in a cerebellum-dependent associative motor learning task, delay eyeblink conditioning, while the central nucleus of the amygdala (CeA) was inactivated bilaterally. Training trials consisted of a pure tone conditioned stimulus (CS) paired with a periorbital stimulation unconditioned stimulus. Multiple tetrode recordings were collected from the cerebellar anterior interpositus nucleus during training sessions in rats given muscimol or vehicle infusions into the CeA. Profound deficits in learning and learning-related neuronal activity during acquisition and retention were observed in rats with amygdala inactivation compared to controls. After the inactivation phase during acquisition, rats were able to reach the same learning criterion as the control group. Short-latency neuronal responses never developed in rats originally trained during amygdala inactivation, but were robust in the controls. Monosynaptic projections from the CeA and parts of the auditory CS input pathway were investigated with anterograde and retrograde axonal tracers. An axonal projection from the CeA to the lateral basilar pontine nuclei was identified. Collectively, these findings suggest that the amygdala may gate auditory CS input to the cerebellum through projections to the pontine nuclei during associative motor learning.

Disclosures: S.J. Farley: None. **J.H. Freeman:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.01/DD19

Topic: F.03. Motivation and Emotion

Support: FAPESP Grant 2014/02296-7

Title: The role of prefrontal cortex and accumbens in the ethanol context-induced relapse to alcohol seeking

Authors: *R. M. LEÃO[1], P. C. BIANCHI[1], P. E. CARNEIRO-DE-OLIVEIRA[1], P. PALOMBO[1], C. S. PLANETA[1], F. C. CRUZ[2];
[1]PANT - Lab. of Pharmacol., Sao Paulo State Univ. - UNESP, Araraquara, Brazil; [2]Inst. of Physics of São Carlos - IFSC, Univ. of São Paulo - USP, São Carlos, Brazil

Abstract: Background: In human addicts and rat models, environmental stimuli in contexts associated with previous drug use can provoke relapse to drug seeking. Specific patterns of sparsely distributed neurons, called neuronal ensembles, have been recently hypothesized to encode learned associations between these drug-associated contexts and drug effects. Methods: We trained male and female rats to self-administer ethanol in context A, extinguished drug-reinforced responding in a distinct context B and assessed context-induced reinstatement under extinction condition in context A or B (control group). After reinstatement test, rats were anesthetized and perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde; brain were remove and coronal sections from prefrontal cortex (PFC) and nucleus accumbens (Acc) were cut. We determined the proportion of dorsalmedial prefrontal cortex (dmPFC) neurons expressing Fos during the reinstatement test by double-labeling for Fos and the neuron-specific protein (NeuN). Results: On test day, re-exposure to the ethanol-associated context (context A) reinstated ethanol seeking behavior in rats (active lever presses=34.78 ± 6.32; n=14 rats), but not reinstated in the rats exposed to the extinction context (context B) (active lever presses=16.61 ± 2.59; n=14). We also observed a tendency of increase (p=0.08) in fos activation in rats exposed to context A (fos/mm2=60,6 ± 9,3; n=5) compared with rats exposed to context B (fos/mm2=38,9 ± 6,6; n=6). Double-labeling for Fos and NeuN indicated that only a small proportion of neurons were activated. In the dmPFC, Fos was expressed in 6,4 ± 0.4% and 5,6 ± 0.86% of all neurons after exposure to the contex A and B, respectively. Conclusions: Our results demonstrate that context-induced reinstatement of ethanol seeking and indicate that dmPFC may be involved in this behavior; meanwhile more rats are necessary to confirm this trend in dmPFC. Future experiments: We will analyze by double-labeling for Fos and NeuN immunohistochemical detection the neural activation in the accumbens (core and shell) and ventral medial prefrontal cortex. Furthermore, we will determined neuronal activation in Acc

brain area projecting to CPF during etanol context-induced tests by measuring double labeling of the retrograde tracer cholera toxin subunit B (CTb; injected in Acc) with Fos.

Disclosures: **R.M. Leão:** None. **P.C. Bianchi:** None. **P.E. Carneiro-de-Oliveira:** None. **P. Palombo:** None. **C.S. Planeta:** None. **F.C. Cruz:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.02/DD20

Topic: F.03. Motivation and Emotion

Support: DA027679

DA038598

Title: Cellular consequences of extracellular signal-regulated kinase signaling in the nucleus accumbens evoked by reward-predictive cues

Authors: M. R. MARKS[1], J. C. MAUNA[1], A. E. ANDERSON[4], *E. THIELS[1,2,3];
[1]Dept. of Neurobio., [2]Ctr. for Neurosci., [3]Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA; [4]Dept. of Pediatrics, Baylor Col. of Med., Houston, TX

Abstract: After repeated exposure to a neutral stimulus paired with a reward, animals learn that the stimulus (conditioned stimulus, CS) predicts the reward. We showed previously that extracellular-regulated kinase (ERK) increases in the nucleus accumbens (NAc) of rats exposed to a food-predicting CS, and that NAc ERK activation is required for the ability of the CS to potentiate reward-seeking behavior (Shifflet *et al.,* 2008). ERK is known to regulate a wide range of cellular and intracellular functions. A potentially relevant intracellular target is the Kv4.2 voltage-gated potassium channel; when phosphorylated by ERK, channel function is reduced and, consequently, cell excitability and neuronal firing increased (Yuan *et al.,* 2002). We previously found that exposure to a CS causes an increase in ERK phosphorylation of the K4.2 channel in the NAc. Here, we wanted to determine whether (1) exposure to a CS causes induction of immediate early genes (IEGs) in the NAc whose expression is sensitive to neuronal activity, and (2) the effect of CS exposure on IEG induction is mediated by ERK. Rats in the experimental group were trained to associate a tone with sucrose pellets; control rats were exposed to the tone only and served to measure basal IEG expression. After 5 days of associative training, all rats were exposed to the tone only (test) to assess the effect of the CS on NAc mRNA levels of the neuronal activity-sensitive IEGs *arc* and *zif268*. Some rats received infusion of the specific ERK inhibitor U0126 into the NAc on one side and vehicle solution into the NAc on the other side shortly before the test. Samples of NAc harvested immediately after the test were analyzed with real-time qPCR. We found that (1) exposure to a CS causes a 3-fold increase

in mRNA level of both *arc* and *zif268* in the NAc but has no effect on the expression of these IEGs in the visual cortex (control region), and (2) the CS-evoked increase in these IEGs' mRNA level in the NAc is abolished in the presence of U0126. These findings indicate that NAc ERK is involved in the regulation of *arc* and *zif268* by appetitive CSs. Together with our previous findings that exposure to an appetitive CS causes an ERK-dependent increase in in Kv4.2 phosphorylation and findings by others that exposure to a CS causes an increase in NAc cell firing (e.g., Day et al, 2006), it is tempting to speculate that the increase in CS-evoked ERK activation causes an increase in NAc cell firing in part through its action on the Kv4.2 channel.

Disclosures: M.R. Marks: None. **J.C. Mauna:** None. **A.E. Anderson:** None. **E. Thiels:** A. Employment/Salary (full or part-time):; National Science Foundation.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.03/DD21

Topic: F.03. Motivation and Emotion

Title: Rapid sucrose sensing in food-restricted mice

Authors: *M. JIN[1], R. FROUSIOS[2], H. LU[2], A. KUO[2], G. SUH[2], R. FROEMKE[2];
[1]NYU Sch. of Med., New York, NY; [2]NYU Sch. of Med., New York City, NY

Abstract: Food restriction is used in many protocols as motivation for animals to perform in behavioral tasks. Here, we offer unconditioned C57BL/6 wild-type mice the choice between two nutritionally-different solutions - sucrose or sucralose - in order to investigate the effects of food restriction on decision-making in rodents. Assays were conducted using short-term water-restricted animals to first establish baseline preferences. The mice were then placed into either food restriction or control groups, in which food-restricted mice were maintained at 80-85% of their initial body weights. In overnight, 2 hour and 1 hour preference tests, food-restricted animals exhibited higher sucrose preference indices compared to the control animals (where 100% preference indicates only drinking from one solution and 50% indicates equal sampling of both sucrose and sucralose). To determine when this preference first develops, we built digital lickometers for each solution and preference tests were performed. In just 10 min upon introduction of the two solutions, food-restricted animals averaged a 68.5% preference for sucrose ($n=11$; $p<0.01$), while control animals averaged a 56.8% preference ($n=9$). Surprisingly, preference for sucrose in food-restricted animals seems to emerge in the first 10 s of the assay, with 73.4% preference for sucrose in food-restricted animals ($n=11$; $p=0.01$) and 47.9% preference in control animals ($n=9$). These preferences were observed in both naïve and experienced animals, suggesting that sucrose preference was not entirely learned by previous

exposure to the two solutions. These results show that food restriction can powerfully control decision-making and motivated behavior based on both sensory experience (taste) and internal state (hunger).

Disclosures: **M. Jin:** None. **R. Frousios:** None. **H. Lu:** None. **A. Kuo:** None. **G. Suh:** None. **R. Froemke:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.04/DD22

Topic: F.03. Motivation and Emotion

Support: DA006886

DA029873

DA023641

NJ06156

Title: Accumbens cue reactivity and affective state in a rat model of binge eating

Authors: ***J. STAMOS**[1], N. BELLO[2], A. PAWLAK[2], A. TYLOR[2], D. QUINTIN[2], K. COFFEY[2], J. KULIK[2], M. WEST[2];
[1]Rutgers, Piscataway, NJ; [2]Rutgers, New Brunswick, NJ

Abstract: Project Summary: Binge Eating Disorder (BED) and Bulimia Nervosa (BN) are both complex disorder with social, behavioral, and self-image components. BN is characterized by repeated binge eating (BE) episodes where the person with BN consumes larger portions of food then they normally would in a discreet period of time. These episodes are followed by unreasonable compensatory behavior which can include self-induced vomiting, excessive exercise, or fasting. BED is characterized with episodes of BE as described for BN but with no compensatory behavior. BED has also been associated with feelings of negative affect and diminished self-worth. In recent years there has been growing interest in the involvement of the Nucleus Accumbens (Nac) in eating disorders such as BN and BED. However due to the complex nature of these disorders a comprehensive animal model for either of them may not be possible. In this study we used an established rat model of BE in order to study the effects of a history of BE on Nac cue processing. Here female Sprague Dawley (SD) rats were used as subjects for the experiment. Subjects were divided into two groups: binge eating (BE) and chow control (CC). The BE group was subjected to a six week treatment where they were twice weekly allowed 30 minutes of unlimited access to a mixture of sucrose and Crisco (10% sucrose by weight). All subjects had unlimited access to standard lab chow and water. Following

treatment all subjects had a 16 microwire array surgically implanted into their right Nac. Following recovery from surgery all animals were subjected to 10 consecutive daily pavlovian sessions in which a tone cue (CS) was pared with presentation of 32% sucrose solution (US). Each session was comprised of 56 trials with a variable interval of on average one minute. Behavioral measures such as number of missed trials and approach latency were measured over days. Neural data was divided into three phases for analysis: cue processing, approach, and consumption. Neural data from each phase was compared with analogues non-cued behavior from the same trial. Furthermore, ultrasonic vocalizations (USV) were recorded during each pavlovian session and were compared to USVs recorded in a baseline session. USVs can be used to give an accurate estimation of a rat's affective state and in this context was used to see if a history of binge eating would affect the animal's affective state. Preliminary behavioral results indicate that BE rats learn the pavlovian task faster than the CC group. USV data indicates a heightened response to the pavlovian chamber compared to baseline sessions in BE animals. Analysis of neural data is still forthcoming.

Disclosures: **J. Stamos:** None. **N. Bello:** None. **A. Pawlak:** None. **A. Tylor:** None. **D. Quintin:** None. **K. Coffey:** None. **J. Kulik:** None. **M. West:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.05/DD23

Topic: F.03. Motivation and Emotion

Support: T32 DA007268

P01 DA031656

T32 DA007267

Title: Performance of a cue-triggered conditioned response elicits greater neural activity in the ventral pallidum when the cue is attributed with incentive salience

Authors: ***A. M. AHRENS**, T. E. ROBINSON, J. W. ALDRIDGE;
Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: In both humans and animals there is individual variation in the degree to which reward-paired cues can bias attention towards them and trigger food- and drug-seeking behavior. These individual differences can be modeled in rats using a Pavlovian conditioned approach procedure, in which a retractable lever (the conditioned stimulus, CS) is repeatedly paired with food reward. In this procedure, some rats begin to approach and interact with the lever when it is present ("sign trackers", STs), while other rats approach the food cup when the lever is present

("goal trackers", GTs). For both STs and GTs the lever acquires predictive value; however, for STs the lever is thought to also gain incentive value, making it attractive and capable of eliciting approach. The goal of the current study was to determine whether neural activity in the ventral pallidum (VP), a mesolimbic structure that encodes the motivational value of cues, reflects the enhanced incentive motivation seen in STs compared to GTs. We used single-unit in-vivo electrophysiology to examine neural firing in the caudal region of the VP during Pavlovian conditioned approach. We used video ratings to conduct a detailed analysis of behavior in STs and GTs, and examined VP neural activity in response to behavioral events characteristic of sign- and goal-tracking, including orienting to the CS, approach to the lever or food cup, and individual instances of contact with the lever or food cup. We found that VP neurons responded to the moment immediately preceding the first contact with the intended target in each trial (the lever for STs and the food cup for GTs). These responses to initial contact were stronger in STs than GTs, both in terms of percentage of responsive neurons and the magnitude of responses. The same cells did not respond to subsequent contact events within the same trial, suggesting that these responses were not related to specific motor actions. In addition, for STs, many of the neurons that responded to lever contact also responded to the moment when rats entered the magazine to retrieve the food pellet. These results suggest that neurons in the caudal VP encode the motivational state that immediately precedes the acquisition of a desired reward, which for the STs includes both the lever itself and the food pellet. Furthermore, these results show that the intensity of this motivational state depends on the degree to which a cue acquires incentive-motivational value.

Disclosures: **A.M. Ahrens:** None. **T.E. Robinson:** None. **J.W. Aldridge:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.06/DD24

Topic: F.03. Motivation and Emotion

Support: NIH-NIDA Grant 11-PAF05335

Title: Motivational activation of dopamine neurons is greater in sign-trackers compared to goal-trackers

Authors: L. FERGUSON[1], A. M. AHRENS[1], L. G. LONG[1], *J. W. ALDRIDGE[2];
[2]Psychology, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: The purpose of this study is to investigate individual differences in the attribution of incentive salience to Pavlovian cues through activation of dopamine neurons. We recorded neural activity in the ventral tegmental area (VTA) in rats performing a Pavlovian approach task

(Flagel et al., 2007). In this behavioral model, a conditioned stimulus (CS) consisting of an illuminated lever is presented for 8 seconds at random intervals followed by delivery of a food reward (unconditioned stimulus, US). All animals learned the predictive nature of the cue (illuminated lever entry into cage). Rodents that preferentially approach the lever itself ("sign-trackers", STs) were compared to animals that predominantly approach the location of reward receptacle ("goal-trackers", GTs). Following training to determine phenotype, rats had tetrodes implanted for neural electrophysiological recordings in VTA. Dopamine cells were characterized by spike waveform shape and firing rate (Roesch et al., 2007) and in 8 sessions, the dopaminergic nature was confirmed by a systemic apomorphine (0.75 mg/kg) test. Electrode bundle placement was confirmed histologically. Firing rates and magnitudes of responses in relation to Pavlovian behaviors, cue presentation, and reward delivery were assessed. We identified 42 dopamine and 48 non-dopaminergic neurons. Overall, 72% of neurons were responsive (85% dopaminergic, 60% non-dopaminergic). GTs and STs both showed responses to the initial (400ms) lever presentation (CS1) and also lever retraction (CS2). However, higher firing rates were sustained during the 8s interaction period only in STs, while they interacted with the lever. Even though GTs interacted just as vigorously with the goal there was no concomitant neural activation indicating that motor differences do not account for the findings. Most responsive neurons in STs were dopaminergic (50% vs. 18%). Further, firing rate magnitudes for both dopaminergic and non-dopaminergic units rates were higher in STs. Neurons outside the VTA were not responsive to the task. These results support an important role for dopamine neurons in the attribution of incentive salience to reward predictive cues and underscore the consequences of potential differences in motivational behavior between individuals. Flagel SB et al. Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. Psychopharmacology (Berl) 191-599-2007 Roesch MR et al. Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. Nat Neurosci 10-1615-2007

Disclosures: **L. Ferguson:** None. **A.M. Ahrens:** None. **L.G. Long:** None. **J.W. Aldridge:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J Wayne Aldridge, Marc Bradshaw, Andrew Klein.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.07/DD25

Topic: F.03. Motivation and Emotion

Title: Effects of prior stress exposure on motivation for sucrose rewards: Possible sex differences

Authors: *E. M. ANDERSON, M. MCWATERS, Z. BOND, N. MULLEN, Z. AHMAD, L. MATUSZEWICH;
Northern Illinois Univ., DeKalb, IL

Abstract: Dysfunction of motivational systems for natural rewards has been implicated in several disorders, including depression, substance abuse, and obesity (Jones, et al., 2013; Volkow & Wise, 2005). Stress is frequently hypothesized as a contributing factor to these disorders in humans and animals. Likewise, sex differences have been found in the prevalence of motivational disorders and in the response to stress, however it is not known if stress alters motivation for sucrose rewards in a sex dependent manner. Therefore, the goal of the current research project was to better characterize the motivational changes to a natural reinforcer following chronic stress exposure in male and female rats. Adult rats were either exposed to chronic unpredictable stress (CUS) for 10 days or handled daily but received no additional stress (CON). One week following the last day of stress, rats were trained to lever press using a fixed ratio (FR) which increased across five days. The rats were then tested on a progressive ratio (PR) schedule to assess motivation for a sucrose reward. Following PR, rats were re-trained on a FR1 for a total of 10 days and then their behaviors were extinguished to 10% of the FR1 responding. The day after extinction criteria was reached, yohimbine was administered to measure stress-induced reinstatement responding. Female rats showed a significantly greater number of responses and rewards earned compared to males in the progressive ratio test ($p<.01$). Prior exposure to CUS did not have a significant influence in PR responding. When re-trained on a FR1 schedule, there was also a sex difference in responding with males making a significantly greater number of responses and earning more rewards compared to females ($p<.05$). Interestingly, female rats exposed to CUS earned a greater number of rewards overall on the FR1 compared to CON females, while males exposed to CUS earned fewer than CON males ($p<.05$). Following extinction, both male and female rats previously exposed to CUS had a significantly greater increase in yohimbine-induced reinstatement compared to CON rats ($p<.05$). Overall, the data suggests that there are sex differences in sucrose motivation, but the impact of prior CUS differs by the testing parameter. Following extinction, prior exposure to CUS in both sexes potentiated responding to the acute pharmacological stressor, yohimbine. The current findings provide insight into the motivational differences between males and females and future research will investigate whether the current findings extend to stimulants, such as methamphetamine.

Disclosures: E.M. Anderson: None. **M. McWaters:** None. **Z. Bond:** None. **N. Mullen:** None. **Z. Ahmad:** None. **L. Matuszewich:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.08/DD26

Topic: F.03. Motivation and Emotion

Title: Reward variety effects on instrumental actions in rats

Authors: *B. HALVERSTADT, H. C. CROMWELL;
Bowling Green State Univ., Bowling Green, OH

Abstract: Humans and animals respond to diversity in food items by increasing intake and appetitive behaviors, and this observed variety effect reflects changes in the motivational value of such rewards. Previous work on the effects of food variety has posed two mechanisms by which these changes in motivation may come about. Variety may slow habituation processes by decreasing exposure to any one food item. Variety effects may be due to incentive contrast, whereby comparisons between items impact their relative value. The current work uses an experimental paradigm with more than one level of variety and a chain of two different instrumental actions in series, rather than a single type of operant response, to expand on what is known about how reward variety affects motivational processes. This study also adds predictive cues about impending outcomes, allowing examination of the potential impact of factors such as preference. We investigated rats' responses to qualitative reward variety presented in different contexts. We used three flavors of sucrose rewards to test rats' patterns of operant responding in three contexts: no, low, and high variety (one, two and three flavors, respectively). We compared response patterns across no, low and high variety contexts, and we compared responses in no variety contexts presented before and after a variety context. We also tested rats' response patterns when predictive cues about upcoming outcomes were added to these conditions. We paired a distinct tone cue to each of the three flavors, which signaled the flavor of the upcoming reward. This study aimed to examine how variety influences motivation. If variety is intrinsically rewarding it would boost the motivational value of all outcomes presented in a variety context irrespective of the original value of any individual outcome. We predict that rats will exhibit a general invigoration of responding in variety compared to no variety contexts, and that the degree of responding will be related to the level of variety (low vs high). If variety is rewarding due to some relative comparison process, it would differentially boost or diminish the value of individual outcomes relative to others when presented in a variety context. We expect that predictive cues about impending outcomes will provide short-term indicators of variety that influence responding based on comparisons to the other outcomes, and that this differential responding may be related to flavor preference. The results of this study have meaningful implications for deepening our understanding of motivational processes in general, as well as for informing potential clinical approaches to dietary disorders.

Disclosures: B. Halverstadt: None. **H.C. Cromwell:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.09/DD27

Topic: F.03. Motivation and Emotion

Title: Chromatin remodeling and gene induction in sign and goal-tracking rats

Authors: *E. HARVEY, K. GUARDADO, J. OCHOA, P. KENNEDY;
Univ. of California Los Angeles, Los Angeles, CA

Abstract: Recent work has identified individual differences in the attribution of incentive salience to discrete and contextual stimuli. When trained in the same Pavlovian appetitive procedure, some animals exhibit approach to a conditioned stimulus while others approach the site of reward delivery. These animals are characterized as sign-trackers and goal-trackers, respectively. Differences between sign- and goal-trackers extend to other behaviors; goal-trackers exhibit stronger context-induced renewal of drug-seeking (Saunders et al., 2014) and greater freezing response to shock paired contextual stimuli (Morrow, Maren, & Robinson, 2011), while sign-trackers undergo more severe discrete cue-induced reinstatement of drug-seeking (Saunders and Robinson, 2011) and exhibit greater cue-conditioned fear (Morrow, Maren & Robinson, 2011). The molecular mechanisms supporting the acquisition and expression of one type of memory over another - contextual versus discrete cue salience - remain largely unknown. Chromatin remodeling can facilitate or suppress memory formation through bidirectional regulation of plasticity related genes (Cortes-Medoza et al. 2013). To investigate chromatin regulation of gene expression in incentive memory formation and behavioral bias, we first trained rats to asymptote in a Pavlovian Conditioned Approach (PCA) procedure known to induce varying expression of sign- and goal-tracking behavior. We then assessed histone modifications associated with transcriptional activation and repression and the induction of activity and plasticity related genes across brain regions implicated in discrete cue and context learning.

Disclosures: **E. Harvey:** None. **K. Guardado:** None. **J. Ochoa:** None. **P. Kennedy:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.10/DD28

Topic: F.03. Motivation and Emotion

Support: NIH Grant AG045380

Title: Age-related differences in licking microstructural indices of incentive motivation and hedonic impact in rats

Authors: *I. A. MENDEZ[1], N. P. MURPHY[1], S. B. OSTLUND[2], N. T. MAIDMENT[1]; [1]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA; [2]Anesthesiol. and Perioperative Care, UCI, Irvine, CA

Abstract: Analysis of licking microstructure may be used to discern the incentive properties from the hedonic impact of food rewards. Specifically, the number of licking bouts a rat voluntarily engages in reflects incentive motivation for food, whereas the average length of bouts once engaged, reflects hedonic properties. While age-dependent differences in food consumption have been reported, the specific processes (i.e. incentive versus hedonic) driving these differences are unclear. To this end, licking microstructure during consumption of sweet solutions was studied in adult (20 weeks old) and aged (21 months old) rats under sated and hungry conditions. Animals were trained to lick for a 0.01% concentration of the non-caloric sweetener, saccharin, under sated conditions. Following training, microstructural licking responses to varying concentrations of saccharin (0.005%, 0.01%, 0.02%, and 0.04%) and sweetened condensed milk (2.5%, 10%, 25%, 50%) were studied under sated and hungry (12 hr food deprivation) conditions using a lickometer. Aged rats emitted significantly fewer licking bouts for 0.01% saccharin during training and across a range of saccharin concentrations while sated, indicating a motivational deficit. When hungry, significantly fewer bouts of licking were again observed in aged rats. In addition, a significant interaction between age and concentration was observed for mean bout length, with aged rats emitting shorter bouts, but only for the 0.005% saccharin concentration. When sweetened condensed milk was used, aged rats exhibited significantly fewer bouts of licking and shorter bout lengths across all concentrations when tested sated. Despite generally attenuated licking, aged rats displayed significantly higher locomotor activity than adult rats across all tests. Together, these findings suggests that the motivational, and in some cases, hedonic impact, of caloric and non-caloric food rewards are attenuated in aged rats. Understanding how aging impacts incentive motivation and reward palatability may inform development of targeted cognitive-behavioral and pharmacological therapies for age-dependent impairments in reward seeking and taking behavior.

Disclosures: I.A. Mendez: None. **N.P. Murphy:** None. **S.B. Ostlund:** None. **N.T. Maidment:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.11/DD29

Topic: F.03. Motivation and Emotion

Support: EMBO ALTF 664-2012

2014 NARSAD Young Investigator Grant

NIH Grant

Title: Bidirectional value coding in the habenula projecting pallidum is essential for optimal decision-making

Authors: *M. STEPHENSON-JONES, S. AHRENS, M. PENZO, A. VAN HUIJSTEE, K. YU, B. LI;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The ability to weigh information about rewarding and aversive stimuli and choose appropriate behavioral actions is essential for survival. Brain circuits including the basal ganglia and the mesocorticolimbic dopamine system subserve such behavioral choice. Recent findings have indicated that the lateral habenula (LHb), and its input from the habenula-projecting globus pallidus (GPh) play a key role in conveying negative motivational signals that can suppress dopaminergic activity and lead to aversion. However, neurons in the GPh and LHb are also inhibited by reward and silencing the LHb is rewarding in mice. While this suggests that the GPh-LHb circuitry may integrate reward and aversive information, how these signals are used to guide behaviour is unknown. To address this question we recorded from GPh neurons, while mice associated different sounds with rewarding and aversive outcomes. As in primates, expectation of an aversive stimulus led to an increase in neuronal firing, while expectation of a rewarding stimulus caused a decrease in neuronal firing. In both cases the neuronal responses were graded depending on the expected magnitude of reward or punishment. Consequently individual GPh neurons can integrate appetitive and aversive stimuli, to bidirectionally encode value. To determine the functional consequence of these firing rate changes we optogenetically activated the GPh selectively during a probabilistic switching task. Activating this pathway decreased the value of an action and led to switching during the task. This suggests that the GPh neurons may encode how “bad” an action is through an increase in firing. Indeed selectively reducing the glutamatergic drive onto these neurons, by blocking synaptic trafficking of GluR4-containing AMPA-Rs, decreased the ability of mice to integrate negative feedback and led to aberrant perseverance in the task. In contrast, optogenetic inhibition of these neurons was sufficient to drive reward-like behavior and increase the value of an action. This suggests that these neurons may also encode how “good” an action is through a decrease in firing. Indeed reducing the GABAergic input to these neurons, by selectively ablating the GABAA receptor γ2 subunit, impaired the ability of mice to integrate positive feedback from rewards. Together our results show that GPh neurons bidirectionally encode the expected value of an action. Without these bidirectional changes mice may not be able to use either positive or negative information to guide behaviour, suggesting that the GPh represents a key node where information of opposing valence is combined to generate a unified value code.

Disclosures: M. Stephenson-Jones: None. **S. Ahrens:** None. **M. Penzo:** None. **A. van Huijstee:** None. **K. Yu:** None. **B. Li:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.12/DD30

Topic: F.03. Motivation and Emotion

Support: NIMH Grant 5R01MH068073

NIMH Grant 1R21MH104718

Title: Examining the involvement of the serotonin 2c receptor in goal-directed motivation

Authors: *M. BAILEY[1], V. WINIGER[4], C. MEZIAS[5], C. WILLIAMSON[2], P. BALSAM[5], E. SIMPSON[3];
[1]Columbia Univ., New York, NY; [2]Psychology, [3]Psychiatry, Columbia Univ., New York City, NY; [4]New York State Psychiatric Inst., New York City, NY; [5]Psychology, Barnard Col., New York City, NY

Abstract: Behavioral assays that isolate distinct components of motivation have determined that patients with schizophrenia and depression often display normal hedonic reactions in the moment, but have a reduction in the willingness to work for goals due to altered effort-reward computations. To date, however, there are no effective pharmacological treatments for specifically alleviating this reduction in willingness to work. Here we describe experiments using mice to explore a potential pharmacological treatment for amotivation. We have employed our battery of operant behavioral tasks to determine what specific aspects of motivation may be enhanced by treatment with a functionally selective 5-HT2c receptor ligand (SB242084). In addition to two widely used assays of motivated behavior, the progressive ratio and the effort based choice task, we also developed a progressive lever hold-down task in which the bar must be continuously depressed for progressively longer durations to obtain reward. This novel assay of motivation differentiates between increases in responding caused by generalized hyperactivity from those produced by increased motivation. SB242084 increases responding on a progressive-ratio schedule of reinforcement in both male and female wild type mice. SB242084 also increases willingness to work in an effort-based choice paradigm. Finally, this increase in responding is not due to hyperactivity because SB242084 also increases how long subjects will hold a lever down to earn rewards, but did not impact efficiency (rewards/lever press) in our progressive lever hold-down assay. Further, a series of experiments using the progressive ratio determined that the drug acts in an acute manner, and the behavioral effects can be reinstated repeatedly as multiple administrations of the drug over time lead to behavioral increases of the same magnitude. Our results suggest that targeting the 5-HT2c receptor can have a selective impact on effort-reward computations underlying action. This manipulation can be used for investigating the neural mechanisms underlying the specific aspects of motivation disrupted by disease.

Disclosures: **M. Bailey:** None. **V. Winiger:** None. **C. Mezias:** None. **C. Williamson:** None. **P. Balsam:** None. **E. Simpson:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.13/DD31

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA015188

NIH Grant MH63649

Title: Optogenetic dissection of neuroanatomical and psychological components underlying amygdala-mediated incentive motivation

Authors: *S. M. WARLOW[1], M. J. F. ROBINSON[2], K. C. BERRIDGE[1];
[1]Psychology, Univ. of Michigan, Ann Arbor, MI; [2]Wesleyan Univ., Middletown, CT

Abstract: We have previously shown that optogenetic excitation of central amygdala (CeA) causes an intensely narrowed preference for a laser-paired sucrose reward over an alternative identical sucrose reward. Similarly, CeA excitation causes an intense preference for laser-paired cocaine over an identical cocaine reward. Here, we aimed to separately examine these effects neuroanatomically and psychologically. We first neuroanatomically assessed if the laser control of cocaine preference is localized to CeA and not to basolateral amygdala (BLA), similar to the sucrose produced localization. Second, we psychologically assessed which of the three psychological components contained in our instrumental preference test contributes most to CeA's laser control of preference: 1) Pavlovian incentive salience ('wanting' the CS) 2) Instrumental habit reinforcement (Stimulus-response habits) or 3) Sucrose/cocaine outcome enhancement (making it a more valued reward). Preliminary findings suggest that the CeA focused preference may be driven more by incentive salience than the other two components. Overall, these tests may help advance our understanding of how excessive motivation in various pathologies is directed towards a single target.

Disclosures: S.M. Warlow: None. **M.J.F. Robinson:** None. **K.C. Berridge:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.14/DD32

Topic: F.03. Motivation and Emotion

Support: NIH Grant 5R01MH063649-12

Title: Optogenetic manipulation of posterior paraventricular thalamic circuits alters chocolate intake

Authors: *K. URSTADT, D. A. ALI, E. R. GRANT, N. M. RABAH, K. C. BERRIDGE; Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Prior studies implicate the posterior paraventricular thalamus (pPVT) in controlling food intake; those data show that pPVT lesions and inhibition with muscimol increase intake. Paradoxically, pPVT excitation with orexin A also increases intake. Therefore, we sought to explore how optogenetic excitation or inhibition of the pPVT may similarly alter intake of normal chow and highly palatable food. Two groups of four adult Long Evans rats received pPVT microinjection of adeno-associated viruses coding for channelrhodopsin or halorhodopsin, and optic fiber implants were aimed at the pPVT, central amygdala (CeA), and nucleus accumbens shell (AcbSh). Rats were tested for one hour in an open field chamber with access to water, chocolate fragments, and chow. Yellow laser-mediated inhibition of the pPVT increased chocolate intake (10.3 +/- 0.8 g) relative to intake without laser (6.4 +/- 1.1 g; p = .049), but blue laser-mediated excitation of the pPVT did not significantly alter intake. Excitation of pPVT to AcbSh projections suppressed chocolate intake (0.9 +/- 0.9 g) relative to intake without laser (4.0 +/- 0.0 g; p = .023), but inhibition of this projection did not significantly alter intake. Manipulations of pPVT to CeA projections did not significantly alter intake. Chow intake remained unchanged in all tests. These data suggest that 1. pPVT optogenetic inhibition may mimic the appetitive effects of muscimol microinjections, and that 2. excitation of the pPVT to AcbSh circuit suppresses appetitive behavior.

Disclosures: K. Urstadt: None. **D.A. Ali:** None. **E.R. Grant:** None. **N.M. Rabah:** None. **K.C. Berridge:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.15/DD33

Topic: F.03. Motivation and Emotion

Title: Junk-food enhances calcium-permeable AMPAR transmission in the nucleus accumbens and cue-induced motivation

Authors: *M. F. OGINSKY, R. C. DERMAN, C. W. NOBILE, C. R. FERRARIO; Univ. of Michigan, Ann Arbor, MI

Abstract: Foods with high sugar and fat content ("junk-foods") are readily available and contribute to obesity. Recent work in people and rodents shows that diet-induced obesity is associated with alterations in brain circuits that mediate reward and motivation. For example, exposure to food-cues increases the fMRI BOLD signal in the nucleus accumbens (NAc) more strongly in obese vs. non-obese people. This enhanced NAc response may be driven in part by glutamate transmission, as AMPARs provide the main source of excitation to the NAc, and AMPARs play critical roles in the incentive-motivational properties of reward-paired cues. Furthermore, the NAc activity mediates cue-induced motivation in non-obese rats, and we recently showed that cue-induced motivation is enhanced in rats susceptible to obesity. These data suggest that alterations in AMPAR function in the NAc may contribute to enhanced motivation for food-cues in obese individuals. However, no studies have examined the effects of diet-induced obesity on NAc AMPAR-transmission, nor is it clear whether interactions between predisposition and consumption of "junk-food" produce different neurobehavioral adaptations in susceptible individuals. Therefore, we examined the effects of "junk-food" exposure on NAc AMPAR-mediated transmission, AMPAR-expression, and cue-induced motivation in obesity-prone (OP) and obesity-resistant (OR) rats. We found that junk-food exposure followed by a deprivation period (14 d) enhanced CP-AMPAR transmission in the NAc core of OP rats. Interestingly, this diet manipulation did not produce differential weight gain in OP vs. OR rats, suggesting that the induction of CP-AMPARs was not due to weight gain per se. Consistent with our observations in selectively bred rats, outbred rats that gained weight during junk-food exposure showed greater increases in NAc GluA1, but not GluA2, surface protein expression compared to rats that did not become obese on the same diet. Together, these data suggest that consumption of "junk-food" enhances NAc CP-AMPAR transmission in susceptible individuals. This differs somewhat from cocaine-induced up-regulation of CP-AMPARs, which requires extended intravenous cocaine exposure and a longer drug free period (~21 d). Additionally, CP-AMPARs mediate cue-induced cocaine-seeking, but their role in motivation for food-cues related to obesity has not been examined. Our preliminary studies show that consumption of "junk-food" enhances cue-induced approach. We are currently investigating the contribution of enhanced CP-AMPAR function to increased cue-induced motivation after "junk-food" exposure.

Disclosures: **M.F. Oginsky:** None. **R.C. Derman:** None. **C.W. Nobile:** None. **C.R. Ferrario:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.16/DD34

Topic: F.03. Motivation and Emotion

Title: Optogenetic activation of the central amygdala generates addiction-like preference for reward despite adverse consequences

Authors: *M. J. ROBINSON[1], R. L. TOM[2], A. AHUJA[2], H. MANIATES[2];
[1]Psychology, [2]Neurosci. & Behavior, Wesleyan Univ., Middletown, CT

Abstract: Drug and behavioral addictions are characterized by focused pursuit of a single reward above all others. Motivation to pursue reward has frequently been associated with structures in the mesolimbic dopaminergic system. The amygdala interacts with key structures in this pathway, including the nucleus accumbens, ventral tegmental area, and substantia nigra, suggesting that it plays a role in reward processing and generating motivation. In this study, we explored how stimulation of the central amygdala (CeA) via optogenetic activation of channelrhodopsin-2 (ChR2) modulates choice, causing specific rewards to be almost compulsively preferred. Rats were trained on an operant task in which they could choose to respond on either of two levers to receive a sucrose pellet. One of the two levers was paired with laser stimulation of the central amygdala upon receipt of the pellet. Rats developed an almost exclusive preference for the laser-paired reward over the otherwise equal unpaired reward. We found that this preference for stimulation-paired reward persists even when a much larger sucrose reward is offered as an alternative, or when this preferred reward is paired with adverse consequence in the form of an electric footshock. We also found that optogenetic activation of the central amygdala does not generate a preference when it is not paired with reward, suggesting that this activation increases the value of reward but is not independently rewarding. Our findings suggest that the central amygdala is involved with assigning increasing value to reward and generating narrowly focused motivation to seek out reward. These results may indicate that mesocorticolimbic dysfunction, particularly in the CeA, can bias motivation and desire to generate addiction-like behavior, a desire that will persist in the face of more rewarding alternatives and adverse consequences.

Disclosures: **M.J. Robinson:** None. **R.L. Tom:** None. **A. Ahuja:** None. **H. Maniates:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.17/DD35

Topic: F.03. Motivation and Emotion

Title: 'Liking' and 'wanting' for a sweet reward in rodents maintained on a prenatal and lifetime junk food diet

Authors: *E. N. LESSER[1], S. J. MI[2], A. ARROYO-RAMIREZ[2], M. J. F. ROBINSON[2];
[1]Wesleyan Univ., Oak Park, IL; [2]Neurosci. & Behavior, Wesleyan Univ., Middletown, CT

Abstract: Obesity rates have shown a dramatic global increase in the last few decades. The increase in availability, palatability and advertising of high-sugar and high-fat junk foods is largely to blame for this trend. The result is a well-documented ever-growing population of overweight and obese individuals. The greatest risk, however, may be for the escalating number of children born to obese and overweight parents and exposed to a junk food diet even before birth. Excessive exposure to junk food from birth may negatively impact typical brain development and disrupt the functioning of motivation and reward pathways. In this study we investigated the effects of a junk food diet composed of chocolate chip cookies, peanut butter, potato chips and chocolate milk powder on cue-motivated behavior, or 'wanting', as well as the effects of diet on hedonic 'liking' for sweet tastes. Tests were carried out in three offspring populations that were exposed to a mash of human junk food, standard chow, or both. All animals were exposed to the assigned diet prenatally through the mother and maintained on the diet throughout life. Properties of 'wanting' for food-paired cues were examined using a conditioned place preference paradigm and Pavlovian conditioned approach (autoshaping). While each group showed a conditioned place preference for a context paired with junk food, the shift in preference appeared to be greatest for chow-fed animals. Similarly, cues predictive of sucrose delivery appeared to elicit less cue-directed behaviors in animals chronically exposed to junk food. Finally, 'liking' was assessed using a taste reactivity paradigm. Animals fed a standard chow diet showed increasing pleasure responses with greater concentrations of sucrose solution (1%, 3%, 9%). In contrast, animals exposed to a junk food diet displayed a blunted sensitivity to increasing concentrations of sucrose, showing similar amounts of 'liking' responses to each sucrose solution. Because the animals maintained on a junk food diet did not gain significantly more weight than the standard chow animals, these findings suggest that there may be neural differences in 'liking' and 'wanting' systems produced by a junk food diet that does not dependent on weight gain.

Disclosures: E.N. Lesser: None. **S.J. Mi:** None. **A. Arroyo-Ramirez:** None. **M.J.F. Robinson:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.18/DD36

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA015188

NIH Grant MH63649

Title: Optogenetic stimulation versus inhibition of orbitofrontal and insular cortical hotspots on hedonic and motivated behaviors

Authors: *D. C. CASTRO, K. C. BERRIDGE;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Recently, our lab has found two novel hedonic hotspots in the cortex where mu opioid or orexin receptor agonist microinjections cause enhanced 'liking' reactions to the taste of sucrose. One of these hotspots is located in rostromedial orbitofrontal cortex (OFC) and the other is localized to caudal insular cortex (IC). Here, in the OFC or IC hotspots, we further probed the nature of these new hotspots by optogenetically stimulating neurons infected with either channelrhodopsin (ChR2) or halorhodopsin (NpHR3.0). After four weeks of incubation, rats were tested on taste reactivity, food intake, self stimulation, operant place preference, and social interactions tests with laser administration. These tests may further our understanding of whether these novel hotspots use generally excitatory or inhibitory mechanisms, and whether their hedonic amplification abilities transfer to other reward-related behaviors.

Disclosures: D.C. Castro: None. **K.C. Berridge:** None.

Poster

358. Reward: Motivational Mechanisms II

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 358.19/DD37

Topic: F.03. Motivation and Emotion

Support: DA015188

MH63649

DA007267

Title: Counteracting hyperpolarization mediated motivation with optogenetic depolarization in the nucleus accumbens shell

Authors: *S. L. COLE, C. I. COLMENERO, N. A. MOSTOVOI, K. C. BERRIDGE;
Univ. of Michigan, Ann Arbor, MI

Abstract: The nucleus accumbens (NAc) shell has been heavily implicated as a site capable of mediating multiple aspects of motivation. One popular hypothesis is that NAc-mediated motivations are produced by inhibition of NAc GABAergic neurons, subsequently disinhibiting downstream targets, such as the lateral hypothalamus, ventral pallidum, and ventral tegmental area, from tonic inhibition, to motivate behaviors. For example, DNQX, an AMPA-glutamate receptor antagonist produces intense eating when injected into the rostral NAc shell. Here, we tested whether the mechanism of DNQX-generated motivation is through passive inhibition of the NAc, by combating DNQX microinjections with optogenetic stimulation. Consistent with previous studies, we demonstrate that DNQX microinjections produce intense eating behavior.

Further, we show that channelrhodopsin (ChR2) depolarization of NAc neurons at the site of microinjection is capable of abolishing DNQX-induced feeding. These findings demonstrate that NAc hyperpolarization is necessary for DNQX-mediated motivation and indicates that NAc inhibition is a key mechanism in motivated behavior.

Disclosures: S.L. Cole: None. **C.I. Colmenero:** None. **N.A. Mostovoi:** None. **K.C. Berridge:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.01/DD38

Topic: F.03. Motivation and Emotion

Support: American Heart Association 15PRE22820024

Title: Functional connectivity of a brain network is disrupted in children with obesity while viewing high-calorie foods

Authors: *B. A. CHODKOWSKI[1], K. D. NISWENDER[2,3], R. L. COWAN[1];
[1]Psychiatric Neuroimaging Program, Dept. of Psychiatry, [2]Div. of Diabetes, Endocrinol. and Metabolism, Dept. of Med., Vanderbilt Univ. Sch. of Med., Nashville, TN; [3]Tennessee Valley Healthcare Syst., Nashville, TN

Abstract: Background: Both central and peripheral signals drive healthy feeding behavior. However, little is known about functional brain differences in childhood obesity. Ubiquitous exposure to images of food strongly influences eating behavior and may contribute to overeating (drive), reduced ability to control eating (executive control), and subsequent obesity. We hypothesize that a disruption in functional connectivity among brain regions associated with reward, drive, and control contributes to obesity. **Methods:** We probed functional connectivity during a food-cue functional magnetic resonance imaging (fMRI) task via psychophysiological interaction (PPI) analysis. PPI analyses assess the change in functional connectivity between two brain regions during a change in psychological state. We acquired fMRI data from 34 children (16 girls; age = 10.3 (±1.3)). Subjects viewed images of nature, food, and a white crosshair on a black background. The psychological context for our PPI analysis contrasted brain activity while viewing high calorie images vs. low calorie images. For the physiological part of our PPI analysis, we separately investigated two pairs of regions: (1) amygdala and nucleus accumbens (NAc); and (2) anterior cingulate cortex (ACC) and NAc. The NAc, the hub of our network, is associated with reward. The amygdala is associated with motivation and incentive (drive); the ACC is associated with response inhibition (executive control). **Results:** We found differences in PPI between healthy-weight and obese children. Among healthy-weight children, as drive PPI

increases, executive control PPI increases ($p \leq 0.01$; $R^2 = 0.37$). Among children who are obese, as drive PPI increases, executive control PPI decreases ($p \leq 0.03$; $R^2 = 0.27$). The slopes of the regression for each group are statistically significantly different ($p \leq 0.0009$; $F = 13.60$). **Conclusions:** Our results show that for healthy-weight children, as the PPI functional connectivity between a drive-associated region and NAc increases, so too does the functional connectivity between a control-associated region and NAc increase. This positive relationship between drive and control connectivity suggests that healthy weight is maintained when control keeps pace with drive. However, for children who are obese, as PPI drive increases, PPI control decreases, suggesting a loss of control. We show that a reward-related brain network is disrupted in children with obesity.

Disclosures: B.A. Chodkowski: None. **K.D. Niswender:** None. **R.L. Cowan:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.02/DD39

Topic: F.03. Motivation and Emotion

Support: NSERC

Title: Examining the reliability of frontal alpha asymmetry both within a day and between consecutive days

Authors: *R. A. HICKS, W. E. MCILROY;
Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Measurement of EEG frontal alpha asymmetry (FAA) has been suggested to provide information about processing of affect. More specifically, left frontal activity is suggested to correspond with approach motivation while right frontal activity is suggested to correspond with avoidance motivation. Consequently, it is implicated as a potential biomarker for depression risk. The long-term focus of this line of work is to determine if state changes evoked by bouts of exercise modulate affect and depression. However, as an important first step it is necessary to confirm the test-retest reliability of FAA over short term intervals. Reliability of FAA has been shown to be modest over periods of a week or greater but the test-retest reliability in short-term conditions has yet to be established. The objective of this study is to quantify the test-retest reliability of FAA both within a day and between consecutive days. Young healthy adults underwent 4 sessions of EEG collection in the morning and afternoon of two consecutive days. EEG collection occurred in a sound proof booth after a 10-minute quiet period. EEG collection consisted of eight 1-minute collections alternating eyes open or eyes closed in two randomized counterbalanced orders (COOCOCCO or OCCOCOOC). EEG data was referenced to linked

mastoid montage. FAA was calculated as the alpha power (8-12Hz) differences between right and left frontal electrodes. Preliminary results show no effect of daytime or significant changes in FAA between days. Participants had a positive FAA value on average suggesting relatively greater left frontal activity. Intraclass correlations show that across the 4 measurements, FAA has acceptable test-retest reliability (> 0.6) when measured within two consecutive days. FAA appears to be a reliable measure in short-term intervals (within and between days). This provides a necessary background on which to evaluate short term state dependent changes, specifically evoked by exercise, on FAA to inform understanding about neurophysiologic links between exercise and (depression) affect.

Disclosures: **R.A. Hicks:** None. **W.E. McIlroy:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.03/DD40

Topic: F.03. Motivation and Emotion

Support: NSERC MSFSS Grant

Title: The role of the mirror system in the audio and visual perception of emotional hand actions

Authors: ***L. MCGARRY**[1,2,3], R. RAMSAY[2], F. A. RUSSO[3], E. S. CROSS[2];
[1]Psychology, Brain and Mind Institute, Univ. of Western On, London, ON, Canada; [2]Psychology, Bangor Univ., Bangor, United Kingdom; [3]Psychology, Ryerson Univ., Toronto, ON, Canada

Abstract: Background: Neural simulation of other people's emotional movements has been hypothesized to underlie our capacity for empathy. Research suggests that the human mirror system (MS) is activated to a greater extent during (a) visual observation of emotional vs. non-emotional body actions, and (b) auditory observation of emotional vs. non-emotional vocalizations. Questions remain regarding how the MS responds during auditory perception of emotional vs. non-emotional nonvocal actions. In addition, it is not clear whether top-down processes play a role in MS engagement. The current fMRI study examined emotional facilitation of the MS towards hand movements in auditory and visual modalities while manipulating top-down engagement. Methods: The current study employed a 2 (Modality: auditory, visual) x 2 (Judgment type: emotional, non-emotional) x 2 (Stimulus type: emotional, non-emotional) factorial design. A localizer task was employed at the beginning of each fMRI session in order to isolate voxels involved in the auditory or visual perception, as well as execution, of hand actions in every participant. Participants observed a series of emotional or non-emotional hand actions (eg., crumpling up a sheet of paper angrily, or calmly turning a book

page), presented via vision or audition. In one condition, they were asked to judge whether the action they just observed was emotional or neutral in nature. In another condition, they made non-emotion based judgments of whether the action they just observed was completed using one hand or two hands. Analysis: A MS-localizer mask was created using a conjunction analysis of brain activity elicited during the localizer task. This consisted of activity involved in viewing and execution, as well as hearing and execution, of hand movements ((Execute ∩ Hear) ∪ (Execute ∩ View)). Effects of the experimental manipulations were then analysed within this mask. Results: There was greater activation towards emotional vs. neutral stimuli in several MS areas, including the inferior frontal gyrus (IFG) towards visual stimuli, and the IFG and supramarginal gyrus towards auditory stimuli. In addition, the auditory modality induced greater responsiveness to positive emotions, whereas the visual modality induced greater responsiveness to negative emotions. The top-down manipulation elicited enhanced MS activation during non-emotional judgments (number of hands required to complete an action). Results suggest that emotional movements facilitate MS functioning, while task manipulations involving simulation may also affect MS responsiveness.

Disclosures: **L. McGarry:** None. **R. Ramsay:** None. **F.A. Russo:** None. **E.S. Cross:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.04/DD41

Topic: F.03. Motivation and Emotion

Support: NIH R01 EY024912

NIH P50 MH103204

NIH P30 EY008098

NIH P41 RR03631

Title: Early reward-related activity depends on cue salience in LIP but not in the amygdala

Authors: *M. L. LEATHERS[1,2], C. OLSON[1,2];
[1]Ctr. for the Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA; [2]Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In monkeys choosing between rewards and penalties, neurons in parietal area LIP respond strongly to cues associated with both large rewards and large penalties (Leathers and Olson, Science 338: 132-5, 2012). In contrast, neurons in the amygdala respond strongly only to cues associated with large rewards (Leathers and Olson, Program No. 206.12, Neuroscience

2014 Abstracts). These observations suggest that firing in LIP reflects cue salience, as determined by association with a potent positive or negative outcome, whereas firing in the amygdala represents cue value. To test this idea, we trained monkeys to make value-based decisions using cues that could not acquire differential associative salience. On every trial, the monkey chose, with a delayed saccade, between a "safe" cue predictive of moderate reward and an "ambiguous" cue predictive either of large reward or of no reward. The ambiguous cue was a morph containing subtly different proportions of a high-value parent image and a low-value parent image. Choosing the ambiguous cue yielded the outcome associated with the more heavily weighted parent image. We manipulated the discriminability of the ambiguous cues so as to maintain the rate of correct choices at 75%. Under this manipulation, cues signaling large reward and no reward were physically so similar to each other that to identify either required allocating voluntary attention to it. This precluded early automatic capture of attention by the cue associated with large reward. Accordingly, we predicted that early activity correlated with the size of the predicted reward would be attenuated in LIP but not in the amygdala. The key analysis concerned trials in which the monkey chose an ambiguous cue placed in the response field. Across these trials, the only factor that varied was the identity of the ambiguous cue, which predicted either large reward or no reward. In LIP, the population signal reflecting predicted reward-size was weak and late. The fraction of individual neurons carrying a statistically significant reward-size signal was no greater than expected by chance. In the amygdala, the population signal reflecting predicted reward-size was stronger and earlier. The fraction of individual neurons carrying a statistically significant reward-size signal was significantly greater than expected by chance. These results support the view that neuronal activity in LIP reflects cue salience whereas neuronal activity in the amygdala encodes cue value.

Disclosures: M.L. Leathers: None. **C. Olson:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.05/DD42

Topic: F.03. Motivation and Emotion

Support: Scottish Rite Charitable Foundation of Canada

Title: Neural responses to emotionally expressive faces are modulated by attention in amnestic mild cognitive impairment

Authors: *L. MAH[1,2], A. TANG[1,3], N. D. ANDERSON[1,4,2], N. P. L. G. VERHOEFF[2], B. G. POLLOCK[2,5];
[1]Rotman Res. Institute, Baycrest, Univ. of Toronto, North York, ON, Canada; [2]Dept. of Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [3]Dept. of Psychology, McMaster Univ.,

Hamilton, ON, Canada; [4]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada; [5]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Neuropathological changes due to Alzheimer's disease (AD) begin years before cognitive symptoms manifest. Although the earliest abnormalities occur within limbic structures that support both emotion and memory, their impact on emotional processing in preclinical AD is poorly understood. We report preliminary results of a functional magnetic resonance imaging (fMRI) study which compared neural responses to emotionally expressive faces amongst individuals with amnestic mild cognitive impairment (aMCI), a prodromal stage of AD, cognitively normal (CN) older adults, and individuals diagnosed with late-life depression (LLD). We predicted group differences in prefrontal-limbic neurocircuitry that mediate emotion regulation. Twelve non-depressed aMCI (M age= 72; SD= 7.1), 9 LLD (M age= 70; SD= 7.1), and 10 CN (M age= 70; SD= 4.9) were scanned using fMRI at 3T while viewing images of faces with happy, sad, fearful, or neutral expressions. Subjects were instructed to attend to and rate either an affective or physical aspect of faces. Functional neural activity was analyzed using Partial Least Squares (McIntosh et al., 1996, Neuroimage 3, 143). Structural equation modeling was used to assess group differences in effective connectivity between the amygdala and ventral anterior cingulate cortex (vACC). A group x rating task x emotion interaction in fMRI data was found. Independent of task condition, greater amygdalar reactivity was observed to happy expressions in CN, and to sad faces in LLD. In contrast, patterns of functional neural activity in response to emotional faces were modulated by task condition in aMCI. aMCI subjects showed greater amygdalar reactivity to happy expressions when attending to a physical aspect of faces, but to sad expressions when attending to an affective aspect of faces. In the latter condition, aMCI was characterized by decreased effective connectivity between the amygdala and vACC relative to CN. These preliminary data are compatible with the hypothesis that the earliest stages of AD may be characterized by alterations in emotional processing. These changes may include greater amygdalar neuroplasticity, as suggested by attentional modulation of amygdalar responses to emotion in aMCI, but not in either CN or LLD. Further work on the role of amygdalar neurocircuitry in the pathogenesis and treatment of AD is warranted.

Disclosures: L. Mah: None. **A. Tang:** None. **N.D. Anderson:** None. **N.P.L.G. Verhoeff:** None. **B.G. Pollock:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.06/DD43

Topic: F.03. Motivation and Emotion

Title: Deep brain stimulation of the mesolimbic system induces cortical responses in limbic and sensorimotor areas in monkeys

Authors: *M. SUZUKI[1,2], T. ISA[3,2], Y. NISHIMURA[3,2,4];
[1]Dept. of Developmental Physiol., Natl. Inst. for Physiological Sci., Okazaki, Japan; [2]Dept Physiol Sci., Grad. Univ. Advanced Studies (SOKENDAI), Okazaki, Japan; [3]Dept. of Developmental Physiol., Natl. Inst. For Physiological Sci., Okazaki, Japan; [4]JST-PRESTO, Tokyo, Japan

Abstract: Depression is one of major psychiatric disorders. Previous studies have reported that individuals with depression have dysfunction of the ventral striatum (VSt). The VSt plays a critical role in processing reward and reward-motivated behavior. Moreover, deep brain stimulation (DBS) of the VSt has been investigated clinically for the novel treatment of individuals suffering from treatment-resistant depression. However, neural mechanism underlying the therapeutic benefit of mesolimbic-DBS remains largely obscure. Furthermore, although the VSt receives dopaminergic inputs related to reward information from the ventral midbrain, such as the ventral tegmental area (VTA) and the medial substantia nigra pars compacta (mSNc), there are few studies that investigating the effect of DBS of the ventral midbrain. To clarify the effect of DBS of the VSt or the ventral midbrain on the cortical activity, electrical stimulation was delivered to the VSt or ventral midbrain (100-500 μA x 3 at 300 Hz)in two monkeys sedated with ketamine. Evoked-cortical responses in the frontal cortical areas including the sensorimotor cortex (SMC) and limbic cortex such as the orbitofrontal cortex (OFC) and evoked responses in upper limb muscles were recorded simultaneously with electrocorticogram and electromyogram, respectively. DBS of the VSt induced neural response in the OFC but not in the SMC. In contrast, DBS of the ventral midbrain induced responses not only in the OFC but also in the SMC, especially in the primary motor cortex (M1). Evoked response in the SMC was stronger than that in the OFC. In addition, DBS of the ventral midbrain induced muscle responses in upper limb muscles. The onset latencies of the evoked muscle responses were longer than those of muscle responses evoked by stimulation of M1. This result suggested that ventral midbrain could modulated the excitability of the descending motor pathways from M1. The present study has provided the idea to understand the neural mechanism underlying therapeutic DBS to the mesolimbic system. DBS of the VSt modulates neural interaction between the VSt and limbic cortex. DBS of the VTA/mSNc modulates both the limbic and motor cortex. Because DBS of the VTA/mSNc directly affects not only limbic area but also descending motor pathways, clinical application of DBS of the VTA/mSNc could be considered for not only treating the mood disorder but also for facilitating the recovery of deficits in the motor system.

Disclosures: M. Suzuki: None. **T. Isa:** None. **Y. Nishimura:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.07/DD44

Topic: F.03. Motivation and Emotion

Support: NIH NIDA Intramural Research Program Grant

Title: Psychostimulant drug administration markedly increases visual stimulus seeking behavior in calorie-restricted rats; underlying neural mechanisms

Authors: *A. TALISHINSKY, S. IKEMOTO;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Administration of psychostimulant drugs augments goal-directed responses, especially in calorie-restricted animals. Our lab has previously demonstrated that chronic calorie-restriction interacts synergistically with D-amphetamine to potentiate visual stimulus-seeking behavior in rats. Recent work has shown that calorie-restriction affects the sensitivity and responsiveness of the mesolimbic dopamine and serotonin pathways. Continuing with this work, we found that chronic calorie restriction increased visual-stimulus seeking potentiated by the psychostimulant methylphenidate, and decreased sensitivity to the selective serotonin reuptake inhibitor (SSRI) fluoxetine in methylphenidate-potentiated visual-stimulus seeking in rats. To further characterize the neural circuitry underlying the interaction of calorie-restricted physiological state and psychostimulant drug action, we have begun using DREADD-mediated neuronal inhibition of brain regions upstream of the mesolimbic dopamine system, including the medial prefrontal cortex and the basolateral amygdala. Our results suggest that chronic calorie restriction alters behavioral responsiveness to both psychostimulants and SSRIs. Potentiated visual stimulus seeking behavior in calorie-restricted rats may be dependent upon input from the basolateral amygdala. Future work will aim to characterize the involvement of specific BLA efferents in potentiating visual stimulus seeking behavior.

Disclosures: A. Talishinsky: None. **S. Ikemoto:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.08/DD45

Topic: F.03. Motivation and Emotion

Support: NIH MH100583

NIH MH096251

Title: Adiponectin in the prefrontal cortex regualtes depression-related behaviors through AdipoR1

Authors: *J. LIU, M. GUO, H. LI, D. ZHAO, J. LIU, X.-Y. LU;
Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Our previous studies have demonstrated that levels of circulating adiponectin, an adipocyte-derived hormone, are reduced in a chronic social defeat stress model of depression and adiponectin-deficient mice are susceptible to developing depression-like behaviors. Furthermore, we have shown that central administration of adiponectin produces antidepressant-like effects. These results support that adiponectin regulates depressive behaviors via a central mechanism (Liu et al., PNAS, 2012). The neural circuits underlying the actions of adiponectin, however, have not been identified. The expression of adiponectin receptor 1 (AdipoR1) in the prefrontal cortex (PFC) suggests a role of this brain area in mediating the functional effects of adiponectin. In this study, we generated mice lacking AdipoR1 specifically in the PFC and found that these mice displayed depressive-like phenotypes including behavioral despair and anhedonia. Infusion of adiponectin into the PFC produced antidepressant-like effects. These results suggest that the PFC is a functional target of adiponectin and AdipoR1 in this brain area mediates adiponection effects on depression-related behaviors.

Disclosures: J. Liu: None. **M. Guo:** None. **H. Li:** None. **D. Zhao:** None. **J. Liu:** None. **X. Lu:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.09/DD46

Topic: F.03. Motivation and Emotion

Support: PAPIIT-UNAM 216214

CONACYT 238744

CONACYT 176916

Title: PVN magnocellular AVP system signals motivation through suppressing LHb functional output during multifaceted stress coping

Authors: *L. ZHANG, V. S. HERNANDEZ;
Physiology, Medicine, Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: The presence of arginine-vasopressin (AVP) containing axons and terminals in lateral habenula (LHb) has long been observed. However, the origin, neurons acted upon, and

functional implications of these projections have been unclear. To address these questions, we used anatomical/immunohistochemical methods, *in vitro* whole-cell recording in LHB, and *in vivo* extracellular single-unit and local field potential (LFP) recordings. These findings were contextualized by juxtacellular labeling and reconstruction of both hypothalamic AVP neurons in paraventricular nucleus (PVN), and medial division of lateral habenular complex in rat. Additionally, we employed two behavioral tests, live-predator exposure (LPE) and forced swimming test (FST), to test for fear and learned helplessness respectively. Here, we show that a direct vasopressin-containing glutamatergic pathway, from AVP-MNN axon collaterals, selectively targets the medial-LHb GABAergic interneuron recorded and juxtacellularly labeled *in vivo*. At the electron microscopy level, AVP+ dense-core vesicles co-localized with small-clear vesicles at synaptic active zone suggesting AVP plays a role in synaptic transmission. Activation of this pathway by physiological osmotic stress led to a sharp increase in theta power recorded LHbM *in vivo* and a significant reduction of psychomotor deficits during innate fear and learned helplessness processing. Fos expression overall in the LHb was significantly reduced. These finding suggest that the magnocellular AVP containing glutamatergic pathway exerts powerful inhibition in the LHb to promote motivational behavior during a multifaceted stress coping.

Disclosures: L. Zhang: None. **V.S. Hernandez:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.10/DD47

Topic: F.03. Motivation and Emotion

Support: NSERC 341600

Title: Contribution of the medial thalamus to social and non-social behavior

Authors: *E. BONNEMA, Y. CHUDASAMA;
Dept. of Psychology, McGill Univ., Montreal, QC, Canada

Abstract: There is substantial evidence that disturbances in learning and memory are associated with a damaged mediodorsal (MD) thalamus (for review, see Mitchell and Chakraborty, 2013, Front. System Neurosci, 9:00037). On account of its strong afferent input from the central nucleus of the amygdala, the MD thalamus has also been implicated in the expression of fear-related emotions. For example, rats with MD lesions have been observed to freeze more in aversive situations (Vanderwolf, 1967, Physio and Beh, 2:339), and mice with MD lesions show increased anxiety in the elevated plus maze (Chaveau et al., 2005, Behav Brain Res, 156:215). The MD thalamus also sends a direct projection to the ventral and orbital prefrontal cortex

known to mediate emotional responses to socially significant stimuli (Beer et al., 2006, J Cog Neursci, 18:871). Unfortunately, there has been no systematic attempt to understand how the MD thalamus contributes to social aspects of emotion. We thus set out to explore the effect of MD thalamic lesions on social emotional behaviour. A group of Long-Evan rats received bilateral excitotoxic lesions of the MD thalamus through *N*-methyl-D-aspartate (NMDA) injections, or received sham control surgery. We then tested the rats on a variety of tasks to measure social and non-social emotional behaviours. First, we found anxiety levels to be in the normal range as both shams and MD-lesioned rats spent similar amounts of time in the open arms of the elevated plus maze (shams: 70.5 seconds, MD: 68.8 seconds). Second, we found that the MD lesions did not affect sociability measures because, like the sham controls, rats with MD lesions readily approached and interacted with a familiar rat (shams: 103.6 seconds, MD: 81.6 seconds) or a stranger rat (shams: 97.2 seconds, MD: 95.8 seconds). However, when the familiar and stranger rats were replaced with familiar and novel objects, rats with MD lesions exhibited a preference for familiar objects (familiar: 162.9 seconds, novel: 53.9 seconds), whereas the sham control rats explored familiar and novel objects for similar amounts of time (familiar: 75.7 seconds, novel: 82.1 seconds). These preliminary data indicate that although rats with MD lesions are sensitive to objects that have never been encountered before, and thus avoid them, they nonetheless readily interact socially with rats, even if those rats are complete strangers. Further analyses and studies will explore the nature of the object avoidance behaviour and the type of behaviours that constitute social interactions.

Disclosures: E. Bonnema: None. **Y. Chudasama:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.11/DD48

Topic: F.03. Motivation and Emotion

Support: JSPS KAKENHI, Scientific Research (B) #25291071

JSPS KAKENHI, Challenging Exploratory Research #26650114

Title: Social facilitation of foraging effort in domestic chicks: functional contribution of the descending pathway from arcopallium to midbrain tegmentum

Authors: *X. QIUHONG[1], T. MATSUSHIMA[2];
[1]Grad. Sch. of Life Sci., [2]Fac. of Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Social facilitation denotes an increment of individual activity that occurs in the presence of one or more conspecifics (Crawford 1939). Since pioneering psychological reports (Zajonc 1965, Clayton 1978), however, the underlying neural mechanisms has rarely been

studied. Ogura and Matsushima reported at the SfN2014 meeting that electrolytic lesion of the midbrain tegmental area resulted in a partial suppression of facilitation, whereas the selective depletion of catecholaminergic neurons by 6-OHDA micro-infusion failed to reproduce the lesion effect. It is thus suggested that some tegmentum pathways other than the ascending dopaminergic projections may be responsible. Arcopallium (Arco, previously archistriatum) is a candidate, as it constitutes one of the major telencephalic descending efferents in the tegmentum. Arco constitutes a heterogeneous structure with multiple functional roles in behavioral control. Arco is assumed to play a "limbic" function (Saint-Dizier et al. 2009, Phillips & Youngren 1971). Arco is also critical for sensori-motor controls (Zeier & Karten 1971), particularly the spatial working memory of sound localization (Knudsen & Knudsen 1996). Furthermore, Arco is involved in the cost-based decision making (Aoki et al. 2006). In this study, we examined effects of bilateral electrolytic lesion to Arco on social facilitation. Chicks were trained in an I-shaped maze equipped with a pair of feeders at both terminals, and grains of millet were delivered at variable intervals. The maze was composed of two parallel lanes separated by a transparent wall. A subject chick was initially placed in one lane and tested in isolation; subsequently it was paired with a companion chick in the parallel lane. Finally the companion was removed and the subject chick was tested in isolation again. Chicks actively shuttled between the two feeders and foraged the delivered food. Pairing significantly increased the running distance, and the increase was supposed to represent the social facilitation. We compared among 3 groups of chicks, (1) Arco lesion, (2) nidopallim lesion control, and (3) sham lesion control, and found that a significant suppression of the social facilitation occurred in the group (1). In all of the chicks in group (1), we also found an increase in κ value (number of pecks per unit gain), suggesting an impaired sensori-motor coordination. The foraging effort in the isolate condition was also suppressed in some chicks, but not significantly. We therefore conclude that (i) descending pathway from Arco to tegmentum plays a critical role in social facilitation, and (ii) Arco is responsible for other functions such as sensori-motor coordination.

Disclosures: X. Qiuhong: None. **T. Matsushima:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.12/DD49

Topic: F.03. Motivation and Emotion

Title: Prosubiculum rather than subiculum heavily projects to medial prefrontal cortex, amygdala, ventral striatum, bed nucleus of the stria terminalis and hypothalamus in mouse

Authors: *S.-L. DING, J. W. PHILLIPS;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Hippocampal formation (HF) plays important roles in motivation, emotion, reward, drug addiction, stress, anxiety and fear. Anatomically these functions have been attributed to subiculum (Sub) and/or hippocampal CA1 region with less consideration of the prosubiculum (ProS). The ProS was originally designated by Lorente de No to the region located between the Sub and CA1 but it has not commonly been used in modern literature on rodent HF. However, a recent comprehensive analysis has revealed that there indeed exists a unique ProS in rodents, as is seen in monkeys and humans. In order to explore connectional evidence for differential functions of the ProS and Sub in mouse the present study investigated and compared the target regions of these two HF subregions defined by unique gene markers. After the anterograde tracer rAAV was injected into intermediate and ventral ProS, heavily labeled axon terminals were observed in the medial prefrontal cortex [mainly infralimbic cortex (IL)], lateral entorhinal cortex, distal CA1, anterior olfactory nucleus (AON), lateral part of lateral septal nucleus (LSN), ventral striatum (VS: nucleus accumbens and olfactory tubercle), amygdala, bed nucleus of the stria terminalis (BNST), anteromedial and paratenial nuclei of the thalamus, and many hypothalamic regions. Much less terminal labeling was detected in AON, amygdala, BNST, and hypothalamus after dorsal ProS injections. In contrast to ProS injections, rAAV injections in the Sub resulted in heavily labeled axon terminals in a different set of brain regions including medial entorhinal cortex, area 29 of the retrosplenial cortex, pre-/para-subiculum, area prostriata, medial LSN, medial mammillary nucleus, anteroventral, laterodorsal and reuniens nuclei of the thalamus. In summary, the present study revealed distinct projections of the ProS and Sub with the former having wider spread projections. The middle and ventral ProS predominantly target the IL, VS, amygdala, BNST and hypothalamus, regions critical for motivation, emotion, reward, drug addiction, stress, anxiety and fear. The Sub mainly projects to the regions important for procession of spatial information such as medial entorhinal cortex, area 29, pre-/para-subiculum, medial mammillary nucleus, anteroventral nucleus of the thalamus. These results also call for careful localization of the ProS and Sub in lesion, injection, stimulation and recording studies in rodents. A detailed comparison of the connectivity between the ProS and Sub will be presented.

Disclosures: **S. Ding:** None. **J.W. Phillips:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.13/DD50

Topic: F.03. Motivation and Emotion

Support: R01MH63291

Title: The hippocampus projects to bed nucleus of the stria terminalis but not the central amygdala nucleus in primate

Authors: J. IOURINETS[1], *J. L. FUDGE[2];
[1]Neurosci., Univ. of Rochester, Rochester, NY; [2]Dept of Neurobio. and Anat., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: The bed nucleus of the stria terminalis (BNST) and central nucleus (CeN) of the amygdala are opposite poles of the 'central extended amygdala', a macrostructure that is best known for its role in responses to aversive stimuli. The extended amygdala is most extensively studied in rat, where afferent inputs to the lateral BNST and CeN are quite similar. Despite this, behavioral and lesion studies suggest differences in lateral BNST and CeN function, with the lateral BNST required for long-lasting fear responses evoked by unconditioned stimuli and contextual cues, whereas the CeN is more involved in immediate fear responses and consolidation of conditioned stimuli. Comparatively little information has been obtained in the nonhuman primate. To help clarify afferent systems to the lateral BNST and CeN, we first focused on hippocampal inputs. Small injections of retrograde tracers were placed in the BNST and CeN, and the distribution of labeled cells in the hippocampus was charted. Results were confirmed with anterograde injections into the hippocampus. BNST injections resulted in a very restricted but dense distribution of labeled cells in rostral CA1' of the hippocampus, mostly anterior to the dentate gyrus. Surprisingly, none of the CeN injections resulted in labeled cells in the hippocampus. None of the anterograde injections in the hippocampus resulted in labeled fibers in the CeN, and only one injection, in the rostral CA1', issued labeled fibers into the BNST. We then analyzed direct hippocampal-BNST and hippocampal-CeN data together with our previous work on hippocampal-amygdala projections and amygdala-extended amygdala projections. Combined tracing studies indicate that the BNST differs from the CeN in receiving direct hippocampal inputs, while the CeN receives only indirect hippocampal influence through amygdala-CeN pathways. In higher species, the rostral hippocampus, including uncal CA1', is greatly expanded. In humans, this region is associated with autobiographical memory storage and retrieval. Our data suggests that autobiographic information from the past is channeled directly to the BNST, perhaps shaping long-lasting responses in conditions such post-traumatic stress disorder, where cues originate in personal memory. Conversely, our anatomic data indicate that in primates, CeN responsivity is largely shaped by direct amygdala inputs, suggesting a greater role in responses to stimuli presented in the 'here-and-now'.

Disclosures: J. Iourinets: None. **J.L. Fudge:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.14/DD51

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01 MH101209

NIH Grant R01 NS247609

NIH Grant R01 MH/NS57414

Title: Subgenual anterior cingulate area 25 corticocortical connections and their interface with distinct classes of inhibitory neurons

Authors: *M. JOYCE, H. BARBAS;
Hlth. Sci., Boston Univ., Boston, MA

Abstract: Cortical area 25 (A25) is located in the ventral and posterior aspect of the subgenual anterior cingulate region. A25 is a limbic type of cortex with less elaborate laminar structure than the six-layered eulaminate areas. Converging evidence suggests that A25 may have a role in emotional regulation and may contribute to the pathology of mood disorders. Using neural tracers in the rhesus monkey, we investigated the largely unexplored corticocortical projections to A25 to study how it interfaces with areas associated with emotion and cognition. Numerous projection neurons directed to A25 were found in the neighboring ventromedial prefrontal cortex (VMPFC) and anterior cingulate cortex (ACC), both of which are linked to emotion and its expression. Some neurons projecting to A25 were also found in the frontal pole (area 10). There were no significant projections from other dorsolateral prefrontal areas, which are associated with cognition. Outside of the frontal lobe, a significant number of neurons directed to A25 were found in the entorhinal cortex, anterior auditory association cortices, and the anterior insula, an area that is associated with interoceptive function. These findings reveal that A25 receives the strongest input from the VMPFC and ACC, which are involved in emotion and are part of the default mode network (DMN), a resting state network that becomes active when subjects are not engaged in specific tasks. In humans, the DMN is thought to become engaged during internal modes of cognition, e.g., when one reflects on the past or plans for the future. We also analyzed the laminar density of functionally distinct inhibitory neurons in A25 based on expression of the calcium-binding proteins Parvalbumin (PV) and Calbindin (CB). CB neurons were significantly denser than PV neurons, and were found mostly in the superficial layers of A25. PV neurons were distributed equally across the superficial and deep layers of A25. The laminar organization of CB and PV neurons in A25 provide information on the interface of pathways with different inhibitory systems in A25, which may be relevant for the switch between task-dependent and default modes of processing.

Disclosures: **M. Joyce:** None. **H. Barbas:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.15/DD52

Topic: F.03. Motivation and Emotion

Support: NIH Grant P30GM32128

Title: Consumption of food calorically high in fat alters reward circuitry and increases "craving-like" behaviors over periods of abstinence

Authors: *R. A. DARLING[1], T. BROWN[2], P. DINGESS[3];
[1]Univ. of Wyoming, Laramie, WY; [2]Sch. of Pharm., [3]Neurosci., Unversity of Wyoming, Laramie, WY

Abstract: Sensory cues indicative of palatable food rewards have been shown to induce a motivational craving state. This can trigger unhealthy eating behaviors. In rats, craving may be assessed by measuring their response on a lever for cues previously associated with a reward. Craving has been shown to increase in intensity progressively over time for such rewards as cocaine and sucrose. The increased craving behavior manifests in rats as an enhancement in lever responding to contingent cues (light and tone) after extended abstinence periods and subsequently coined, *incubation of craving*. Our lab has previously observed an incubation of craving effect in response to a high-fat reward (HF) following 2 h access training sessions. However, this effect was also observed in our standard chow controls. Previous literature has shown a difference in neuroadaptations following 6 h extended-access compared to 2 h access. Therefore, we repeated our experiments with 6 h training sessions. We tested rats for their responses to the contingent cues following 30 days of abstinence (CE30) from HF. Our results indicate that lever pressing for HF increases from one day of abstinence (CE1) to CE30 (44.3 ± 4.9 vs. 79.3 ± 10.4, $p<0.05$, n=15). In the 6 h training paradigm, cues paired with standard chow pellets do not incubate to the same magnitude as the high-fat group but the relative magnitude of incubation was comparable between the two dietary conditions (24.0± 2.9 vs. 58.6 ± 5.2, $p<0.05$, n= 16). Current ongoing experiments are being conducted to evaluate whether there are differences in structural and synaptic plasticity, which parallel the changes in craving behavior. We conclude that extended-exposure to cues paired with HF undergo an incubation of craving effect, which may contribute to maladaptive food seeking behaviors.

Disclosures: R.A. Darling: None. **T. Brown:** None. **P. Dingess:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.16/DD53

Topic: F.03. Motivation and Emotion

Support: NIDA grant DA022340

NIDA IRP

Title: Mapping of monosynaptic cannabinoid type 1 receptor inputs into dopamine neurons of the ventral tegmental area

Authors: *V. KASHTELYAN[1], C. A. MEJIAS-APONTE[3], M. MORALES[3], J. F. CHEER[1,2]; [1]Dept. of Anat. and Neurobio., [2]Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Intramural Res. Program, Neuronal Networks Section, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: The mesolimbic dopamine (DA) pathway is critically involved in reward-seeking and appetitive behaviors. It originates in the ventral tegmental area (VTA) of the midbrain and projects rostrally to provide profuse DA innervation to terminal regions, in particular the nucleus accumbens. Impaired DA release within the mesolimbic system is a hallmark of schizophrenia, depression and drug abuse. The activity of DAergic cells in the VTA is potently modulated by the endocannabinoid system (ECS). The ECS influences neuronal activity through presynaptic inhibition of neurotransmitter release. The cannabinoid type 1 receptor (CB1R) is the main receptor involved in this signaling pathway. Here, we identify afferents that control firing of DAergic cells in the VTA by means of signaling at CB1Rs. Using DAT::Cre transgenic mice, we first injected two AAV viruses into the VTA. The viruses infected DA cells with an avian retroviral receptor, the mammalian rabies glycoprotein and a fluorescent tag. Mice were later transduced with a pseudorabies virus in which the rabies glycoprotein has been removed and replaced with a fluorescent protein. The pseudorabies binds specifically to DA cells expressing the avian retroviral receptor and utilizes the rabies glycoprotein in DA cells to infect cells that form monosynaptic, classical synapses with DA neurons. Once we identified monosynaptic inputs to DA neurons in the VTA, we performed a combination radioactive in-situ hybridization and immunohistochemistry experiment. We were able to visualize cells that were infected by the pseudorabies virus and cells that express CB1R mRNA. For each brain region in which we found rabies infected cells, we performed a cell count of the pseudorabies infected neurons and the pseudorabies neurons expressing CB1R mRNA to determine the ratio of afferents to DA neurons that are and are not under the control of the ECS. Our results identify discreet populations of VTA DA afferents that signal through the ECS. This confirms that VTA DA neurons are under the control of multiple input regions, which can engage ECS signaling to change activity patterns of dopamine neurons in an activity-dependent negative feedback fashion.

Disclosures: V. Kashtelyan: None. **C.A. Mejias-Aponte:** None. **M. Morales:** None. **J.F. Cheer:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.17/DD54

Topic: F.03. Motivation and Emotion

Support: CRSNG/NSERC

Title: Intra-Accumbens blockade of tyrosine-related kinase B (TrkB) receptors using ANA-12 modulates emotional behaviors and biochemical signaling following a repeated stress regimen in male Wistar rats

Authors: *H. PLAMONDON, I. AZOGU;
Dept Psychol, Univ. Ottawa, Ottawa, ON, Canada

Abstract: Elevated levels of brain-derived neurotrophic factor (BDNF) and its primary receptor, tyrosine-related kinase B (TrkB) in the nucleus accumbens (Nac) shell have been reported following stress, which has been linked to anxiogenic and pro-depressive mood. Recently, the selective TrkB antagonist, ANA-12, was developed which prevents the binding activation of TrkB by BDNF, thus inhibiting processes downstream of TrkB signaling. Four groups of male Wistar rats (n=10 /group) were implanted with guide cannulas in the NAc shell and treated with ANA-12 or vehicle to assess the effects of repeated stress on the activity of BDNF/TrkB in the mesolimbic system, and whether the inhibition of this system can affect anxiety and motivational behaviors. They underwent a ten-day repeated stress/no stress regimen, in which stress regimen involved alternating restraint and forced swim stressors. Rats were later tested in the elevated plus maze, social interaction test, and the forced swim test. In addition, post mortem levels of CRH and vGluT2 within the PVN and BLA regions, as well as Orexin A and ΔFosB in the LH, perifornical area and CA3 of the hippocampus were determined. Findings show that ANA-12 significantly reduced anxiety-like behavior in the EPM and increased social interaction in stressed animals. However, both stress groups performed uniformly in measures of swimming and immobility in the forced swim test. Immunohistochemical results show decreased CRH and vGluT2 following repeated stress in the parvocellular portion of the PVN and in the BLA in ANA-12 stressed rats. Of interest, ANA-12 had intrinsic actions to increase orexin A-ir and Fos B-ir, to the same levels as that observed in the vehicle-treated stress rats. Notably, ANA-12 when injected in stressed rats restored orexin-A and Fos B levels to that found in control (non-stressed) animals. Overall, these data suggest that ANA-12 represent a good tool in understanding the effects of TrkB activation within the mesolimbic system following repeated stress exposure.

Disclosures: H. Plamondon: None. **I. Azogu:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.18/DD55

Topic: F.03. Motivation and Emotion

Support: CIHR Grant 491746

Title: Grey matter differences are predicted by variation in the ADRA2b gene

Authors: *M. R. EHLERS[1], D. J. PALOMBO[2], D. J. MUELLER[3], A. K. ANDERSON[4], R. M. TODD[1];
[1]Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [2]Boston Univ., Boston, MA; [3]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [4]Cornell Univ., Ithaca, NY

Abstract: A common genetic polymorphism in the ADRA2b gene, which codes for the noradrenergic α2b receptor, has been found to predict individual differences in emotional processing in humans. Previous research suggests that the deletion variant has *in vivo* effects similar to that of a noradrenergic antagonist putatively leading to increased extracellular norepinephrine. Deletion carriers have been shown to have greater emotional enhancement of memory than noncarriers and a higher susceptibility to intrusive memories following trauma (de Quervain et al., 2007). During memory encoding deletion carriers show higher amygdala activation (Rasch et al., 2009) and greater attentional bias for emotionally salient stimuli (Todd et al., 2013). ADRA2b deletion carriers also perceive emotional stimuli as more perceptually vivid, an effect driven by increased activation of valuation network hubs proposed to be directly modulated by locus coeruleus noradrenergic activity. That is, in deletion carriers emotionally enhanced vividness is associated with greater activity in the amygdala and ventromedial prefrontal cortex (VMPFC), which modulate visual cortex activity (Todd et al., 2015). Given the accumulating evidence for a role of the ADRA2b polymorphism in affective processing, we hypothesized differences in cortical and subcortical grey matter volume between deletion carriers and noncarriers. In the present study Caucasian participants were recruited from the University of Toronto as part of a collaborative study investigating genetic influences on attention and memory. Participants were 18 - 35 years old and screened for a history of brain injury, learning disabilities and psychopathology. Saliva samples were collected in order to genotype participants for ADRA2b as well as other genes linked to influences of emotion on attention and memory. T1-weighted magnetic resonance images were obtained for a subset of participants. Structural scans of 72 participants (37 deletion carriers) were subjected to cortical and subcortical segmentation using FreeSurfer. Results revealed larger amgydala volume for ADRA2b deletion carriers compared to noncarriers. This anatomical difference provides a putative neural mechanism for deletion carriers' advantage in emotionally enhanced perception and memory reported in the literature. In addition, grey matter volume in other key nodes of valuation networks was investigated. The results demonstrate that individual variations in norepinephrine availability are not only associated with altered affective processing, but are also reflected in anatomical differences.

Disclosures: M.R. Ehlers: None. **D.J. Palombo:** None. **D.J. Mueller:** None. **A.K. Anderson:** None. **R.M. Todd:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.19/DD56

Topic: F.03. Motivation and Emotion

Support: NSERC CGSM

Title: Caudal nucleus accumbens core inactivation results in decreased appetitive motivation and increased aversive motivation in response to conditioned cues

Authors: *L. M. HAMEL, T. THANGARASA, R. ITO;
Univ. of Toronto, Toronto, ON, Canada

Abstract: The nucleus accumbens is a site of integration of positively and negatively valenced information and action selection. Differentiations in valence processing have been found along the rostrocaudal axis of this structure, in assessments of both unconditioned motivation and in approach-avoidance decision making in response to conditioned cues. A previous study revealed that disruption of activity in the caudal accumbens core via a GABA receptor agonist resulted in a bias toward aversion in response to conflicting cues signaling both reward and punishment. In the current study we sought to determine whether this finding was mediated by enhanced avoidance, diminished approach motivation, or a combination thereof. Rats were trained to associate visuo-tactile cues with appetitive sucrose, aversive foot-shocks and neutral outcomes. In order to assess motivation in response to conditioned cues, separate tests were conducted which measured exploratory preference for the arms containing the appetitive versus neutral cues, and the aversive versus neutral cues. Animals receiving GABA receptor agonists in the caudal core region spent a decreased proportion of time in the aversive arm as compared with control animals, as well as a decreased proportion of time in the appetitive arm as compared with controls. The results suggest that the caudal nucleus accumbens core mediates both appetitive and aversive motivation in response to conditioned cues, and that the behaviour observed after inactivation of this region during decision-making tasks likely results from a composite of effects on the processing of both valences.

Disclosures: **L.M. Hamel:** None. **T. Thangarasa:** None. **R. Ito:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.20/DD57

Topic: F.03. Motivation and Emotion

Support: NIH 5T32MH087004-05

NIH 2T32MH087004-06

NIH 5R01MH090264-05

Title: The role of lateral habenula orexin signaling in aggressive behavior

Authors: *M. FLANIGAN[1], H. ALEYASIN[1], S. GOLDEN[2], M. HESHMATI[1], M. PFAU[1], G. HODES[1], S. RUSSO[1];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: The neuropeptides orexin-A and orexin-B are produced from the precursor peptide pre-pro orexin solely by neurons in the lateral hypothalamic area (LHA) and have been implicated in a broad spectrum of behaviors including reward, arousal, and response to stress. Developmental knockouts of the pre-pro orexin gene display attenuated defense responses when presented with an intruder mouse, suggesting that the orexin system may be important in aggressive behavior. To investigate the role of orexin in aggression, we first measured expression of orexin peptide and its receptors throughout the brain in aggressive and non-aggressive CD1 mice following exposure to an intruder mouse. Aggressors displayed marked increases in orexin signaling between the LHA and the lateral habenula (lHb), a brain region implicated in the motivational components of aggression. Acute I.P. injection with 30 mg/kg EMPA, a brain-penetrant selective orexin receptor 2 (OxR2) antagonist, significantly decreased aggression. AAV-shRNA-mediated knockdown of OxR2 in the lHb also decreased aggression, further strengthening the importance of OxR2 in this behavior and providing a previously undefined behavioral role for orexin signaling in the lHb.

Disclosures: **M. Flanigan:** None. **H. Aleyasin:** None. **S. Golden:** None. **M. Heshmati:** None. **M. Pfau:** None. **G. Hodes:** None. **S. Russo:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.21/DD58

Topic: F.03. Motivation and Emotion

Support: F30MH105199

R01MH101180

5T32NS007433

5T32GM008208

Title: Differences in regulation of medial vs. lateral dopamine neurons in rodents by lateral habenula and infralimbic prefrontal cortex: relevance to the chronic mild stress model of depression

Authors: *J. L. MOREINES[1,2], Z. OWRUTSKY[1], A. A. GRACE[1];
[1]Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Med. Scientist Training Program, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Although serotonin has been classically associated with major depressive disorder (MDD) emerging data from both clinical and animal model studies have implicated the dopamine (DA) system in the reward and motivational impairments core to the disorder. Specifically, we found in two models of MDD a significant down-regulation in DA neuron population activity that correlated with depression-like symptoms. Human studies have also implicated two other regions in the pathophysiology of MDD, Brodmann Area 25 (homologous to rodent infralimbic prefrontal cortex, ILPFC) and the lateral habenula (LHb). In this study, we explored how the ILPFC and LHb may contribute to the down-regulation of DA neurons in the unpredictable chronic mild stress (UCMS) model of depression. We performed single-unit extracellular recordings of identified ventral tegmental area (VTA) DA neurons from anesthetized rats following LHb or ILPFC pharmacological activation. We then assessed whether inactivation of the ILPFC or LHb in animals that previously underwent 5-7 weeks of the UCMS (which features a hypodopaminergic phenotype) would restore normal DA system population activity in this model. We found that activation of either ILPFC or LHb in normal rats potently suppressed VTA DA neuron population activity, albeit in different patterns. ILPFC activation primarily affected medial VTA DA neurons, whereas LHb activation had a greater effect on central and lateral VTA DA neurons. Similarly, in rats that underwent UCMS (which impacts primarily medial VTA DA neurons), only ILPFC inactivation restored VTA DA neuron population activity to normal levels, whereas LHb inactivation had no restorative effect on DA neuron population activity. These data suggest that the ILPFC and LHb might regulate different subpopulations of DA neurons within the mesolimbic DA system. This striking pattern of differential modulation could explain the unique restorative capacity of ILPFC inactivation in reversing abnormal DA system hypoactivity in the widely used UCMS animal model of MDD. More generally, these results suggest that in humans, abnormal activity in BA25 vs. the LHb may drive different types of dopamine-related pathological states in patients with MDD.

Disclosures: J.L. Moreines: None. **Z. Owrutsky:** None. **A.A. Grace:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.22/DD59

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA034975

Title: Rewarding effects of pain relief require endogenous opioid activity in anterior cingulate cortex

Authors: *E. NAVRATILOVA, C. QU, N. LAUDE, C. KRAMER, D. MESKE, E. LEMISTER, J. Y. XIE, X. YUE, M. HEIEN, F. PORRECA;
Univ. of Arizona, Tucson, AZ

Abstract: Clinical and preclinical investigations suggest that relief of pain engages brain emotional and motivational circuits. Relief of pain may thus be considered as a reward. We have shown previously in rodent pain models that relief of pain with non-opioid treatments (e.g., peripheral nerve block or spinal clonidine) activates the mesolimbic dopaminergic reward circuit and elicits relief-motivated behavior (conditioned place preference, CPP). Blockade of dopaminergic signaling in the nucleus accumbens (NAc), part of the reward circuit, prevents pain relief induced CPP. Furthermore, endogenous opioid signaling in the anterior cingulate cortex (ACC), an area encoding pain aversiveness, is required for both pain relief induced CPP and NAc dopamine release. Whether systemically administered analgesics such as gabapentin (Neurontin), used clinically for the treatment of neuropathic pain, produce their pain relieving effects by stimulating release of endogenous opioids in the ACC is not known. In this study, we used male Sprague Dawley rats with spinal nerve ligation (SNL) as a model of chronic neuropathic pain and investigated behavioral and neurochemical consequences of pain relief following intravenous administration of gabapentin. Pain relief motivated behavior was evaluated using the conditioned place preference (CPP) paradigm. Dopamine release in the NAc was assessed in freely moving animals using *in vivo* microdialysis. To measure endogenous levels of opioid neurotransmitters (methionine- and leucine-enkephalin) in the ACC, we developed an online-preservation method for improved microdialysis recoveries and quantification of these peptides by Mass Spectrometry. Our studies demonstrate that in rats with neuropathic pain, gabapentin reversed SNL-induced tactile hypersensitivity, produced CPP and elicited release of dopamine in the NAc. Blockade of opioid signaling in the ACC prevented both gabapentin-mediated CPP and dopamine efflux in the NAc. Preliminary assessments of endogenous opioid levels indicate altered opioid neurotransmitter activity in the ACC of neuropathic rats. These findings provide support for a critical role of endogenous opioid activity in the ACC in chronic pain states. Release of endogenous opioids in the ACC appears to be a general mechanism for relief of pain unpleasantness and for rewarding/motivational features of pain relief. Selective engagement of brain cortical opioid circuits may allow the identification of new molecular targets and development of novel therapies to alleviate pain. Supported by DA034975. The authors have no conflict of interest.

Disclosures: **E. Navratilova:** None. **C. Qu:** None. **N. Laude:** None. **C. Kramer:** None. **D. Meske:** None. **E. Lemister:** None. **J.Y. Xie:** None. **X. Yue:** None. **M. Heien:** None. **F. Porreca:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.23/DD60

Topic: F.03. Motivation and Emotion

Support: NIH F32 Grant MH100888 to KMN

National Center for Responsible Gaming Early Stage Investigator Award to KMN

NIH T32 Grant MH015174 to KMN

NIH R01 Grant MH082773 to CB

NIH R37 Grant MH068542 to RH

Title: Impulsive action and impulsive choice are distinct behavioral components with different underlying neural substrates

Authors: *K. M. NAUTIYAL[1,2], S. WANG[2], M. M. WALL[2], P. D. BALSAM[2,3], C. BLANCO[2], R. HEN[2];
[1]Neurosci., Columbia Univ., New York, NY; [2]Psychiatry, Columbia Univ. and New York State Psychiatric Inst., New York, NY; [3]Psychology, Barnard Col., New York, NY

Abstract: Impulsivity is a core feature of many psychiatric disorders including substance use disorder, pathological gambling, attention deficit hyperactivity disorder, and personality disorders. Acting without forethought and the inability to withhold responding are two behavioral phenotypes of impulsive behavior that are referred to as impulsive choice and impulsive action, respectively. The degree to which impulsive choice and impulsive action represent distinct or related subcomponents of impulsivity is unknown, as is the extent to which they have overlapping neural substrates. In order to test the hypothesis that impulsive action and impulsive choice represent dissociable behavioral constructs with distinct neural circuits, we collected a number of behavioral measures of impulsivity in a single cohort of mice. We included mice with whole-brain knockdown of serotonin 1B receptor (5-HT1BR) expression given its known role in modulating impulsivity from human gene association studies and animal models. Using a tetracycline operator (tetO)/tetracycline-dependent transcriptional silencer (tTS) system, we investigated the effect of both whole-life knockdown and adult-rescue of 5-HT1BR. The absence of 5-HT1BR expression caused increased impulsivity in the differential reinforcement of low-rate responding (DRL) and Go/No-Go operant paradigms, representing a

deficit in impulsive action. Interestingly, this was reversed with adult rescue of the receptor. However, there was no significant effect of 5-HT1BR expression on the discounting rate in the delayed discounting (DD) or probabilistic discounting (PD) operant tasks used as measures of impulsive choice. Further analysis using an exploratory factor analysis (EFA) revealed a good-fitting two-factor model with the latent factors representing impulsive action and impulsive choice (RMSEA<0.001, CFI=1.0, TLI=1.1). Specifically, DRL and Go/No-Go measures loaded onto one factor, while DD and PD loaded strongly onto the other. Loadings of all indicators were >0.4 and the two factors were not significantly correlated. Finally, a multiple indicator multiple causes (MIMIC) analysis revealed that 5-HT1BR expression and sex were significant covariates in the two-factor model. Male mice had significantly higher levels of impulsivity on both factors, while 5-HT1BR knockdown resulted in significantly increased impulsivity in the impulsive action factor only. Overall, the data support the conclusion that impulsive action and impulsive choice are distinct behavioral phenotypes with dissociable biological influences.

Disclosures: **K.M. Nautiyal:** None. **S. Wang:** None. **M.M. Wall:** None. **P.D. Balsam:** None. **C. Blanco:** None. **R. Hen:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.24/DD61

Topic: F.03. Motivation and Emotion

Support: NS-23805

Title: What is novel about the "deep frontal nucleus"?

Authors: *D. ROBY, K. P. PARSLEY, D. S. ZAHM;
Pharmacol. and Physiological Sci., St. Louis Univ., Saint Louis, MO

Abstract: A dense cluster of round and ovoid neurons in the deep frontal lobe of the rat becomes uniformly and intensely labeled following injections of retrograde tracer into the ventral tegmental area (VTA), consistent with the accumulation of dense, fine gauge labeled axons in the VTA after the cluster is injected with anterograde tracer (Soc Neurosci Abstr 442.05, 2014). This aggregation of neurons, tentatively called the deep frontal nucleus (DFN), sits astride the rostralmost tip of the accumbens and the anterior commissure just in front of it. Its pyriform shape (in frontal sections) stems from structures that bound it medially, laterally and below - respectively, the deep layer of the infralimbic cortex (IL), fornix minor and subependymal sheath of the olfactory ventricle. Despite being wedged between the IL, medial orbital and dorsal peduncular areas of frontal cortex, DFN neurons appear to be not pyramidal - suggesting non-cortical character. Here we addressed whether the efferent projections of the DFN differ from

those of cortical structures abutting it by injecting the DFN with one anterogradely transported axonal tracer, either Phaseolus vulgaris-leucoagglutinin or biotinylated dextran amine, and, in the same subjects, injecting adjacent cortical areas with the other tracer. Series of cases with injections into only the DFN or nearby cortex were also evaluated. Labeled outputs from both the DFN and IL in the present material resembled those earlier reported for IL (Hurley et al., JCN 308:249-76, 1991), which included to piriform and agranular and dysgranular insular cortex, olfactory tubercle, accumbens, ventromedial caudate-putamen, preoptic region and lateral and posterior hypothalamus, thalamic reuniens, submedius and paraventricular nuclei, VTA, substantia nigra compacta, periaqueductal gray, laterodorsal tegmental nucleus and parabrachial nucleus. However, labeling in piriform and insular cortices and their extensions into the temporal lobe were much denser and widespread after DFN as compared to IL injections, which produced anterograde labeling that was everywhere more patchy and focal and extended densely into some areas, such as the supramammillary region, not innervated by DFN. In contrast, olfactory bulb was labeled after DFN but not IL injections. Thus, despite clear similarities in distributions of DFN and IL outputs, their general character differs. Nowhere is this more striking than in the VTA, to which the DFN sends an exceedingly fine gauge, dense projection that, in the immunofluorescence image, starkly contrasts with coarser, sparser projections from the IL. We anticipate that EMs in preparation will further this distinction.

Disclosures: D. Roby: None. **K.P. Parsley:** None. **D.S. Zahm:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.25/DD62

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA025679

NIH Grant DA038114

Title: Evidence of functional connectivity between the nucleus accumbens and ventral pallidum in hedonic processing

Authors: *C. CHAN[1], D. S. WHEELER[2], R. A. WHEELER[2];
[2]Biomed. Sci., [1]Marquette Univ., Milwaukee, WI

Abstract: The nucleus accumbens (NAc) and its major output target, the ventral pallidum (VP), are both involved in reward learning and hedonic processing. Rewarding gustatory stimuli generally reduce the firing rates of NAc neurons while increasing the firing rates of caudal VP neurons, leading to the proposal that rewarding stimuli disinhibit the VP by reducing GABA-ergic input. However, we have observed considerable regional heterogeneity in the encoding of

rewarding and aversive stimuli in the VP. Here we examined the firing patterns of anterior VP neurons during exposure to palatable and unpalatable stimuli, as well as following the drug-induced devaluation of palatable stimuli. In this design, an otherwise palatable saccharin solution elicits a negative affective state as it comes to predict cocaine administration. Adult male rats, implanted with stainless steel microwires for electrophysiological recordings, were intraorally infused with a 0.15% saccharin solution (0.2 ml delivered over 6 sec/trial for 45 trials) that predicted a cocaine injection (20 mg/kg, ip). Rats received daily taste-drug pairings for 7 days. On the test day, rats were presented with the cocaine-predicting saccharin and VP neuronal activity was recorded. Unlike reports of recordings in the posterior VP, most phasically active neurons recorded in the anterior/mid VP reduced firing rate in response to a palatable gustatory stimulus. Interestingly, these responses are also modulated by NAc activity. To examine this functional relationship we pharmacologically inhibited the NAc and measured the neural activity of VP neurons while rats were presented with palatable stimuli. Adult male rats were implanted with guide cannulas in the NAc, bilateral chronic stainless steel microwires for electrophysiological recordings in the VP, and intraoral catheters for sucrose delivery. Following NAc microinfusion of the GABAA agonist muscimol, GABAA antagonist bicuculline or vehicle, animals were intraorally infused with a 20% sucrose solution (0.2 ml/trial for 20 trials) to assess hedonic responses. Then animals were allowed to nosepoke for the same sucrose solution at a FR1 schedule to assess motivated behavior. Consistent with prior investigations, VP activity encoded rewarding taste stimuli, and this encoding was altered by pharmacological manipulations of the VP. These results characterize a functional connectivity from the NAc to VP that modulates the encoding of rewarding taste stimuli. Ongoing studies are further characterizing the heterogeneity of responses in the VP by contrasting these results with more posterior VP recordings.

Disclosures: C. Chan: None. **D.S. Wheeler:** None. **R.A. Wheeler:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.26/DD63

Topic: F.02. Animal Cognition and Behavior

Support: MIUR Prin 2008, 200894SYW2

Toscana Life Sciences Foundation Orphan_0108 program

Title: A rabies virus based approach to map serotonergic neurons innervating different brain structures

Authors: *M. PASQUALETTI[1,2], A. BERTERO[1,2], A. BIFONE[2];
[1]Univ. of Pisa, Pisa, Italy; [2]Ctr. for Neurosci. and Cognitive Systems, Inst. Italiano di Tecnologia, Rovereto, Italy

Abstract: Serotonergic neurons are part of one of the most widely distributed neural systems in the mammalian brain (Lauder and Bloom, 1974). Serotonergic neurons form the raphe nuclei in the brain stem, and are organized in distinct nuclei (B1-9) that project to the whole central nervous system. Consistently with such a broad innervation, serotonin is involved in a wide range of physiological processes including the control of appetite, sleep, memory, mood, stress and sexual behavior (Veenstra-Vanderweele et al, 2000). Several studies using anatomical tracing methods and anterograde viral tracers have led to the hypotheses of a topographic organization of the serotonergic system, with different projections from the caudal, median/central and dorsal raphe neurons to specific target districts in the rostral brain (Muzerelle et al. 2014). Experimental evidence suggests that clusters of serotonergic neurons within the raphe nuclei may have distinct functional properties, but the complex organization of serotonergic neuron projections remains poorly understood. The aim of the present study is to map at a finer scale the organization of serotonin neurons projecting to different target areas, thus contributing to understanding the functional role of specific serotonergic neuronal subpopulations. To this end, we used a Tph2::GFP knock-in mouse model, in which serotonergic neurons are clearly labeled by the expression of GFP (Migliarini et al, 2013), and the retrograde recombinant rabies viral tracer to map the serotonergic neurons innervating different brain structures. Moreover, we developed a conditional GFP expressing mouse model, in which the reporter is maintained under the transcriptional control of the Tph2 gene and activated upon an flp mediated somatic recombination, to map the organization of serotonergic neuron subgroups sharing common targets in the brain.

Disclosures: **M. Pasqualetti:** None. **A. Bertero:** None. **A. Bifone:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.27/DD64

Topic: F.02. Animal Cognition and Behavior

Support: 1R01DA0327327

Title: Anatomical characterization of GABAergic projections to the mouse rostromedial tegmental nucleus

Authors: J. L. THOMPSON[1], *T. C. JHOU[2];
[1]Med. Univ. of SouthCarolina, Charleston, SC; [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: The rostromedial tegmental nucleus (RMTg) is a recently characterized brain structure necessary for encoding aversive stimuli and promoting avoidance behaviors. The magnitude of presynaptic glutamate release onto RMTg neurons is implicated in promoting avoidance or inhibiting responding to aversive and appetitive stimuli, respectively. However, it is unknown if RMTg neurons receive GABAergic inputs from distal regions, and if they are implicated in the inhibition of RMTg neurons seen during the presentation of reward-predictive cues. Here, we sought to characterize GABAergic inputs to the RMTg by retrogradely labelling RMTg afferents in VGAT-cre mice crossed to ZsGreen. We injected CTB (0.2%, 74 nL) unilaterally into the mouse RMTg (from bregma, mm; AP: -4.1ML: +1.1, DV (from skull): -4.75, at a 10 degree angle). Brains were sectioned and immunolabeled for fluorescent CTB using a biotinylated secondary antibody and fluorescent streptavidin. Analysis of CTB-labeled neurons were restricted to slices containing the hypothalamus. Several retrogradely labeled neurons negative for Vgat were observed in the lateral and medial regions of the hypothalamus, lateral habenula, and zona inserta as previously reported. Cells colabeled for Vgat and CTB were also found in these regions. A considerable portion of colabelling was found throughout the lateral hypothalamus, near the border of the premammillary nucleus and dorsal to the fornix. We observed the highest density of RMTg-projecting GABAergic neurons in the ventral portion of the zona inserta near the border of the lateral hypothalamus. Although the full extent to which GABAergic neurons project to the RMTg is unclear, a significant portion of RMTg-projecting neurons in hypothalamic regions appear to be GABAergic. How this population of neurons controls behaviors regulated by the RMTg remains to be elucidated.

Disclosures: J.L. Thompson: None. **T.C. Jhou:** None.

Poster

359. Structural and Functional Neurocircuitry of Motivation and Emotions

Location: Hall A

Time: Monday, October 19, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 359.28/DD65

Topic: B.07. Synaptic Transmission

Support: FONDECYT Grant 1141170

Anillo Grant ACT-1109

Title: *In vivo* recording, identification and reconstruction of adult mouse ventral tegmental area (VTA) GABAergic neurons

Authors: *C. A. GONZALEZ-CABRERA[1], T. MONTERO[2], P. HENNY[2];
[1]Dept. de Anatomia, Facultad De Medicina Univ. Católica De Chile, Santiago, Chile; [2]Anatomia Normal, Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: GABAergic neurons of the ventral tegmental area (VTA) intermingle with dopaminergic neurons and, according to previous evidence, can regulate the activity of dopaminergic neurons through local projections. The present work aims to describe the innervation that VTA GABAergic neurons provide to neighbour dopaminergic neurons. In anaesthetised adult male mice, we recorded and labeled VTA single neurons using the juxtacellular technique. Following brain fixation and histological processing, neurobiotin-labeled neurons were identified as GABAergic by the presence of the vesicular inhibitory amino-acids transporter (VIAAT, also known as VGAT) in local axon terminals. We then examined appositions established between neurobiotin-labeled boutons and tyrosine hydroxylase positive (TH+) structures, and confirmed those contacts as synaptic using further staining for the scaffolding protein gephyrin. Preliminary results show that VTA GABAergic neurons leave axon collaterals in the VTA and that they intermingle with TH+ structures. By extending this analysis in a larger number of neurons, as well as to other type of local synaptic afferents (e.g. glutamatergic), this project aims to define the microcircuitry of the VTA.

Disclosures: **C.A. Gonzalez-Cabrera:** None. **T. Montero:** None. **P. Henny:** None.